Assessing variability in yield performance and nutritional quality of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) genotypes under drought conditions

by

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in Discipline of Crop Science

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PREFACE

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

The contents of this work have not been submitted to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Prof. Alfred Oduor Odindo (*Supervisor*)

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DECLARATION 1: PLAGIARISM

I, **Takudzwa Mandizvo**, declare that:

(i) the research reported in this thesis, except where otherwise indicated or acknowledged, is my original work

(ii) this thesis has not been submitted in full or in part for any degree or examination to any other university

(iii) this thesis does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons

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(v) where I have used material for which publications followed, I have indicated in detail my role in the work

(vi) this thesis is primarily a collection of material prepared by me, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included

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My role in each paper and presentation is indicated. The * indicates corresponding author.

Chapter 2

Mandizvo T*., Odindo A.O., Mashilo J. (2021) Citron Watermelon Potential to Improve Crop Diversification and Reduce Negative Impacts of Climate Change. Sustainability 13. DOI: https://doi.org/10.3390/su13042269

Chapter 3

Mandizvo T*., Odindo A.O., Mashilo J. (2022) Nutrient composition and physical properties of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) seeds are related to seed coat visual appearance. South African Journal of Botany. DOI: https://doi.org/10.1016/j.sajb.2022.02.015

Chapter 4

Mandizvo T*., Odindo A.O., Mashilo J., Magwaza L.S. (2022) Drought tolerance assessment of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) accessions based on morphological and physiological traits. Plant Physiology and Biochemistry. DOI: https://doi.org/10.1016/j.plaphy.2022.03.037

Chapter 5

Mandizvo T*., Odindo A.O., Mashilo J., Sibiya J., Beck-Pay S.L. (2022) Phenotypic Variability of Root System Architecture Traits for Drought Tolerance among Accessions of Citron Watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey). Plants 11. DOI: https://doi.org/10.3390/plants11192522

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ABSTRACT

Research is needed to investigate the potential of Neglected Underutilized Crop Species (NUCS) such as citron watermelon, to increase crop diversity and mitigate the effects of prolonged drought because of climate change. Little is known about citron watermelon's food quality attributes (seed popping yield, nutritional value, and lignin content). In addition, there is a need to understand the agro-morphological, physiological and biochemical characteristics associated with drought tolerance in citron watermelon. Therefore, the objectives of this study were: (1) to assess citron watermelon genotypes for food quality attributes (popping yield, chewability and nutritive value) of seeds based on visual appearance, (2) to screen citron watermelon accessions for drought tolerance using morphological and physiological traits, (3) to study the root system architecture of citron watermelon accessions and identify drought-adaptive root traits for cultivar improvement under water-stressed environments and (4) to reveal how citron watermelon responds to combined stress (water deficit and high temperature) with respect to growth, water status, reserve mobilization and metabolite partitioning at seedling stage.

The first study determined whether citron watermelon seed's nutrient composition and physical properties are related to the visual appearance of seed coat. Brown and red-coloured seeds have a higher popping yield than dark-coloured seeds with poor popping ability and are prone to burning during roasting. Seed coat thickness was closely related to hemicellulose contents and cellulose across all seed coat colours. High hemicellulose, cellulose and lignin contents were found in dark and red seeds associated with thick seed coats and increased chewing strength than white seeds. From a nutritional perspective, dark and red seeds were good sources of Cu, Zn, nitrogen and sulfur than brown seeds. Dark and brown seeds were good Mg sources, whereas dark and red seeds were vital sources of potassium.

The second study determined variation in drought tolerance among South African citron watermelon landrace accessions for selection and use as genetic stock for drought-tolerance breeding in this crop and closely related cucurbit crops such as sweet watermelon. The forty citron watermelon accessions evaluated showed varying levels of drought tolerance based on morphological and physiological traits. These allowed five distinct groupings, namely: A (highly drought-tolerant), B (drought-tolerant), C (moderately drought tolerant), D (drought-sensitive) and E (highly drought-sensitive) based on various drought tolerance indices. The following accessions (WWM02, WWM-05, WWM-09, WWM-15, WWM-37(2), WWM-39, WWM-41 (A), WWM-46, WWM-47, WWM-57, WWM-64, WWM-66, WWM-68 and

WWM-79) were categorized as highly-drought tolerant and accessions WWM-03, WWM-08, WWM-14, WWM-21, WWM-33, WWM-35(1), WWM-35(2), WWM-67 and WWM-76 as drought tolerant. These are useful genetic stocks for improving drought tolerance in this crop and related cucurbit crops, including sweet watermelon.

The third study examined citron watermelon accessions' root system architecture and identified drought-adaptive root traits for cultivar improvement under water-stressed environments. The study showed that plasticity and biomass allocation shift according to genotype, presumably to optimise the use of limited resources. The study found significant phenotypic variation in root architecture among citron watermelon accessions that may relate to differences in water uptake. The following traits of root system architecture (RSA) (total root length, root system width, convex hull area and total root volume) were associated with drought tolerance. Further, RSA traits such as root dry mass and root shoot mass ratio were highly correlated with root branch count, root system depth, total root length and leaf number. These traits are useful selection criteria for breeding and developing water-efficient citron watermelon accessions for cultivation in drought-prone environments.

The fourth study identified multiple abiotic stress-induced modifications in different phytosterols (campesterol, sitosterol and stigmasterol) in the seedling axis (embryonic leaf and root) of genetically distinct citron watermelon accessions. Detailed evaluation of phytosterols was done and the effects of the changes observed in stressed plants were discussed.

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Distinctively, I treasure my loving parents, **Mr. C Mandizvo** and **Mrs. M Mandizvo** for all their invaluable sacrifices of time and resources so I can get an education. To the rest of my family (with special mention to my brother and friend **Dr. T Mandizvo**) and friends who have been very supportive and encouraging, thank you all for the love and prayers.

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Chapter 1: Introduction

1.1 Background

Citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) is a multipurpose cucurbit crop grown as food and fodder crop in many parts of Africa (Bultosa et al., 2020; Mashilo et al., 2021). The fresh and dried leaves are cooked to prepare leafy vegetables consumed with staple food such as maize and sorghum porridge(Pasandide et al., 2018). The fresh and dried vines are used as fodder for animals, the seeds are extracted from the fruit, sun-dried and roasted as seed snacks, thus providing nutrition for key macro-and-micro-nutrients such as calcium, magnesium, zinc, iron and copper (Mandizvo et al., 2022). The matured yellow and orange-fleshed fruits are a rich source of phytochemical compounds (linoleic acid, oleic acid, palmitic acid and stearic acid) (Nkoana et al., 2021). The crop's nutritional value, coupled with its tolerance to drought, makes it a crop of choice to achieve food security.

Erratic rainfall patterns and prolonged dry spells resulting from climate change threaten food security due to crop yield losses. The fact that the population is increasing (projected to reach 9.8 billion people by 2050) (Dillard, 2019; Skaf et al., 2020) will compound food production in a changing climate and remain a major challenge in developing countries, particularly in Sub-Saharan Africa. Cultivation of neglected underutilised crop species (NUCS) such as citron watermelon could potentially mitigate the short-term effects of climate change (poor erratic rainfall and prolonged dry spells), contributing to long-term food security by adding to the basket of crop species that farmers can select from, thus enhancing crop diversification in smallholder farmer fields.

Smallholder farmers cultivating many NUCs such as citron watermelon often plant these crops in areas characterized by various biotic (pests and diseases) and abiotic (drought and poor soils) stresses, further compounded by the effects of changing climate (Mabhaudhi et al., 2017; Mugiyo et al., 2022). Besides citron watermelon being well adapted to harsh abiotic stresses, there is no work/documentation to map the areas suitable for their cultivation. Under the prevailing conditions (erratic rainfall), crop suitability maps of citron watermelon must be developed for future production. Furthermore, little is known regarding citron watermelon (C3 plant) ability to tolerate water/drought stress.

Many years of cultivation and selection for desirable fruit and horticultural qualities have narrowed the genetic base of different vegetable crops, among them watermelon. Most South

African watermelon cultivars share a narrow genetic base, making them highly susceptible to biotic (diseases and pests) and abiotic stressors (drought, salinity, heat and cold temperatures) (Levi et al., 2017; Levi et al., 2001). Crop sensitivity is further increased due to many years of domestication in non-arid regions, resulting in the lack of vital traits that allow natural sustainability under extreme conditions in sweet watermelon cultivars.

Citron watermelon (*Citrullus lanatus* var. *citroides*) landraces are known for their unique phenotypic diversity in growth habits, fruit and seed traits. Citron watermelon landraces grown and maintained by subsistence farmers can provide valuable germplasm to select for (1) abiotic stress (drought) tolerance, (2) low lignin content in seeds and (3) palatable (chewable) seeds. There is, therefore, a need to identify traits available in citron watermelon germplasm, particularly for the desires above by farmers, processors and consumers, and for enhancing biotic and abiotic stress tolerance in watermelon cultivars.

1.2 Problem statement

Drought is a major problem among smallholder farmers in sub-Saharan Africa (SSA). Farmers face the challenge of erratic rainfall patterns and have limited water resources (incapability to install modern irrigation facilities). This problem is exacerbated by (i) the effects of climate change leading to prolonged dry spells and (ii) farmers dependence on a few crops with a narrow genetic diversity. This means that the smallholder farmers are affected because yields are low, leading to food insecurity and poor livelihoods. In recent years there is increasing interest in using NUCS such as citron watermelon to improve yield, food and nutrition security under drought. Therefore, research is needed to investigate the potential of NUCS such as citron watermelon to increase crop diversity and mitigate the effects of prolonged drought because of climate change. Little is known about citron watermelon's food quality attributes (seed popping yield, nutritional value, and lignin content). In addition, there is a need to understand the agro-morphological, physiological and biochemical characteristics associated with drought tolerance in citron watermelon.

1.3 Justification

The cultivation of underutilized crops improves agricultural biodiversity to buffer against crop vulnerability to drought and would provide the quality of food and diverse food sources to address food and nutritional insecurity. Instead of using their food, water, topsoil, and massive amounts of land, and energy to raise livestock, subsistence farmers could, for instance, grow neglected underutilised crop species, well adapted and nutritious crops. Therefore, combining staple crops and neglected crops in developing countries would feed more people nutritiously with efficient use of resources, improve long term soil fertility, and create economic opportunities, all of which would provide a path towards breaking the poverty (SDG 1) and hunger cycle (SDG 2) in a changing climate (SDG 13). Though citron watermelon is drought tolerant, there may exist variation for drought tolerance, emphasizing the need to identify and select better, highly tolerant, and well-adapted varieties. Additionally, most neglected crops, including citron watermelon, exhibit a broad gene pool that could be exploited to improve key yield-influencing attributes and nutritional quality.

1.4 Aim of the study

The study seeks to generate knowledge/information on citron watermelon and its potential to add to crop diversification, mitigate climate change effects in smallholder agricultural systems, and understand the mechanism underpinning drought tolerance in C3-xerophytes

1.5 Research questions

i. What are the differences in food quality attributes of citron watermelon seeds associated with seed coat colour?
ii. How do water deficit and heat modulate seedling growth and reserve mobilization in citron watermelon?
iii. What are the “rules of response” developed by citron watermelon to cope with environmental variability (wet-dry soil profiles) regarding root foraging?

1.6 Specific objectives

The specific objectives of the study are:

i. to assess citron watermelon genotypes for food quality attributes (popping yield, chewability and nutritive value) of seeds based on visual appearance
ii. to screen citron watermelon accessions for drought tolerance using drought indices and phenes analyses to select promising lines for use in breeding for drought tolerance
iii. to study the root system architecture of citron watermelon accessions and identify drought-adaptive root traits for cultivar improvement under water-stressed environments
iv. to reveal how citron watermelon responds to water deficit and high temperature with respect to growth, water status, reserve mobilization and metabolite partitioning at seedling stage

1.7 Thesis structure

Chapter 1: **Introduction**

This chapter offers an overview of the current trends in citron watermelon production from a global to regional scale, followed by a review of the current challenges in citron watermelon production. The research aims, objectives, problem statement and justification of the study are also outlined in this chapter.

Chapter 2: **Citron watermelon potential to improve crop diversification and reduce negative impacts of climate change**

The chapter presents a comprehensive review of the literature on drought adaptation in *Citrullus lanatus* spp. (C3 xerophytes), using a systematic review approach. The review discusses the potential of citron watermelon in adding to crop diversification, alternative food uses, and potential by-products. It analyses the role of SSA farmers as key actors in conserving citron watermelon germplasm and biodiversity. A summary of key findings and knowledge gaps for further research are summarized.

Chapter 3: **Nutrient composition and physical properties of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) seeds are related to seed coat visual appearance**

With a hypothesis that seed coat colour could be linked with physical, mechanical, biochemical and ultrastructural properties that may account for food quality — this chapter gives an in-depth report on food quality attributes (roasting quality, eating quality (chewability) and nutritional value) of forty genetically distinctive citron watermelon seed accessions.

Chapter 4: **Drought tolerance assessment of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) accessions based on morphological and physiological traits**

This chapter report the screening of 40 citron watermelon landraces for drought tolerance under restrict water management, using physiological parameters (net photosynthesis, stomatal conductance and intrinsic water-use efficiency), morphological parameters (root traits) and drought tolerance indices (DTI).

Chapter 5: **Phenotypic variability of root system architecture traits for drought tolerance among accessions of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey)**

This chapter unties the “rules of response” developed by citron watermelon to cope with wet-dry soil profiles in rhizotrons.

Chapter 6: **Phytosterols augment endurance against interactive effects of heat and drought stress on biochemical activities of *Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. Ex Greb)**

Water deficit and heat are the main abiotic stresses to which plants are exposed due to global warming. Seedling establishment is a vulnerable developmental transition that plays a critical role in crop production; we carried out in vitro experiments to reveal how seedlings of the citron watermelon respond to water deficit and heat with respect to growth, water status, reserve mobilization, hydrolase activity and metabolite partitioning, including non-structural carbohydrate availability and phytosterols profile.

Chapter 7: **General discussion and conclusion**

This is the general discussion and includes discussing the findings from experimental chapters and their implications. The chapter provides the conclusions and recommendations for future research.

References

Bultosa G., Molapisi M., Tselaesele N., Kobue-Lekalake R., Desse Haki G., Makhabu S., Sekwati-Monang B., Seifu E., P. Nthoiwa G. (2020) Plant-based traditional foods and beverages of Ramotswa Village, Botswana. Journal of Ethnic Foods 7. DOI: 10.1186/s42779-019-0041-3.

Dillard H.R. (2019) Global food and nutrition security: from challenges to solutions. Food Security 11:249-252. DOI: 10.1007/s12571-019-00893-3.

Levi A., Jarret R., Kousik S., Patrick Wechter W., Nimmakayala P., Reddy U.K. (2017) Genetic Resources of Watermelon, in: R. Grumet, et al. (Eds.), Genetics and Genomics of Cucurbitaceae, Springer International Publishing, Cham. pp. 87-110.

Levi A., Thomas C.E., Wehner T.C., Zhang X. (2001) Low Genetic Diversity Indicates the Need to Broaden the Genetic Base of Cultivated Watermelon. HortScience 36:1096-1101. DOI: 10.21273/hortsci.36.6.1096.

Mabhaudhi T., Chimonyo V.G.P., Chibarabada T.P., Modi A.T. (2017) Developing a Roadmap for Improving Neglected and Underutilized Crops: A Case Study of South Africa. Front Plant Sci 8:2143. DOI: 10.3389/fpls.2017.02143.

Mandizvo T., Odindo A.O., Mashilo J. (2022) Nutrient composition and physical properties of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) seeds are related to seed coat visual appearance. South African Journal of Botany 145:405-419. DOI: 10.1016/j.sajb.2022.02.015.

Mashilo J., Shimelis H., Ngwepe R.M. (2021) Genetic resources of bottle gourd (*Lagenaria siceraria* (Molina) Standl.] and citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.): implications for genetic improvement, product development and commercialization: a review. South African Journal of Botany. DOI: 10.1016/j.sajb.2021.10.013.

Mugiyo H., Chimonyo V.G.P., Kunz R., Sibanda M., Nhamo L., Ramakgahlele Masemola C., Modi A.T., Mabhaudhi T. (2022) Mapping the spatial distribution of underutilised crop species under climate change using the MaxEnt model: A case of KwaZulu-Natal, South Africa. Climate Services 28. DOI: 10.1016/j.cliser.2022.100330.

Nkoana D.K., Mashilo J., Shimelis H., Ngwepe R.M. (2021) Nutritional, phytochemical compositions and natural therapeutic values of citron watermelon (*Citrullus lanatus* var. *citroides*): A Review. South African Journal of Botany. DOI: 10.1016/j.sajb.2020.12.008.

Pasandide B., Khodaiyan F., Mousavi Z., Hosseini S.S. (2018) Pectin extraction from citron peel: optimization by Box-Behnken response surface design. Food Sci Biotechnol 27:997-1005. DOI: 10.1007/s10068-018-0365-6.

Skaf L., Buonocore E., Dumontet S., Capone R., Franzese P.P. (2020) Applying network analysis to explore the global scientific literature on food security. Ecological Informatics 56:101062.

Chapter 2: Citron watermelon potential to improve crop diversification and reduce negative impacts of climate change

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Abstract

Citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) is an underexploited and under-researched crop species with the potential to contribute to crop diversification in Sub-Saharan Africa. The species is cultivated in the drier parts of Southern Africa, mainly by smallholder farmers who maintain a wide range of landrace varieties. Understanding the molecular and morpho-physiological basis for drought adaptation in citron watermelon under these dry environments can aid in the identification of suitable traits for drought-tolerance breeding and improve food system resilience among smallholder farmers, thus adding to crop diversification. This paper reviews the literature on drought adaptation of *Citrullus lanatus* spp. (C3 xerophytes), using the systematic review approach. The review discusses the potential role of citron watermelon in adding to crop diversification, alternative food uses, and potential by-products that can be processed from the crop, and it analyzes the role of Sub-Saharan African farmers play as key actors in conserving citron watermelon germplasm and biodiversity. Finally, the review provides a summary of significant findings and identifies critical knowledge gaps for further research.

Keywords: Abiotic stress; *C. lanatus* var. *citroides*; Drought adaptation; Food security; Underutilized crops

2.1 Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) belonging to the *Cucurbitaceae* family originated in Southern Africa (Chomicki et al., 2020). It is a facultative xerophyte following the C3 photosynthetic pathway (Akashi et al., 2001; Levi et al., 2017). The citron watermelon plant is a vine creeper with herbaceous stems up to 3 m long. Young stems and leaves are densely woolly, while the older parts become hairless. The leaves are herbaceous, sometimes unlobed, but usually 3-lobed. Both female and male flowers are on the same plant (monoecious) (Ngwepe et al., 2019). The fruits are formed in different shapes (subglobose, indehiscent globose, ellipsoid, or oblong) and can be up to 200 mm in diameter. The rind of ripe fruit is hairless and smooth with different colours, usually mottled with irregular longitudinal bands (Janick et al., 2007). The flesh is firm and white, green-white or yellowish. The seeds are dicotyledonous, and typically red, white, or mottled in seed coat colour.

Citron watermelon is mainly produced in Southern Africa (Levi et al., 2017). Clustered data FAOSTAT (FAOSTAT, 2019) on melons (including cantaloupes) production show that production has declined in Southern Africa from 1990 to 2017 by approximately 44%. The decline in production is attributed to pests and diseases, drought, and poor agronomic practices. Although, until the beginning of the 1980s, cultivation of citron watermelon was specific to countries in Southern Africa, other regions have understood the potential and benefits of the crop. Consequently, both the research and production of citron watermelon have been growing steadily worldwide (Ngwepe et al., 2019).

The young tender leaves of citron watermelon can be cooked as green vegetable, while mature fruit flesh is mixed with maize meal to prepare porridge (Pasandide et al., 2018). When eaten fresh, the flesh has a non-bitter and blunt taste and often used to prepare preserves by adding sugar. The fleshy pulp contains pectins, which are processed to make perfect preserves. Citron watermelon fruit and vines is a valuable livestock feed during drought (Alam and Rahman, 2014). The seeds can be dried, roasted, and eaten or ground into flour to make condiments. However, despite being a nutrition source, farmers habitually discard citron watermelon seeds, sparing a few to plant in the next season (Gusmini et al., 2004).

Promoting the production and use of under-researched crops such as citron watermelon could offer potential solutions to mitigate climate change's negative impacts (crop failure, hunger, and malnutrition) (Chivenge et al., 2015). This will contribute towards achieving the Sustainable Development Goals (SDGs) of the 2030 agenda, such as SDG 2 (zero hunger) and other interconnected goals such as SDG 1 (no poverty) and SDG 13 (climate change) (Gil et al., 2019).

The crop can add to crop diversity, boost food security and local economies, strengthen rural development, and promote sustainable land-use. The inclusion of measures to encourage crop diversification among smallholder farmers (who focus on growing few crops), is crucial in ensuring a broad food base and balanced nutrition for populations (rural and urban) in developing countries. Therefore, crop diversification beyond over-reliance on a few food crops is vital in achieving food security.

Despite its potential contribution to food security, both national and international research systems have overlooked citron watermelon and other local and indigenous crops because governments and policy-makers do not see their value as food or cash crops. Consequently, governments hardly prioritize resources (funds) to promote their research (Chivenge et al., 2015). The impact of having policy and government support for agricultural research is evident from the support given to the staple food crops (rice, wheat, maize, and beans), which dominate human diets and have had their yields and nutritional values boosted over the years through breeding. For example, these staple food crops have had their genomes mapped to the level of individual base pairs (Mabhaudhi et al., 2019). There is a need to focus on local or indigenous crops that research programmes have previously neglected because of their potential to add value and contribute to food security and improved livelihoods for smallholder farmers.

Research on previously neglected indigenous crops is increasingly being recognized and is receiving continental attention in recent times. For example, in 2011, the New Partnership for Africa's Development (NEPAD) committed to lead a consortium of companies, scientific and government bodies to sequence, assemble, and annotate the genomes of 100 important Africa's neglected food crops (Shepard, 2014). This consortium, African Orphan Crops (AOC) (*africanorphancrops.org*), aimed to train African scientists in plant breeding techniques to breed and improve the sequenced under-utilized crops. These will allow African farmers to grow highly nutritious, productive, and robust crops, creating surpluses for the market.

Citron watermelon has received relatively little research attention as one of the neglected crops on the AOC list. Therefore, the current knowledge on citron watermelon's potential to improve crop diversity and contribute to improved livelihoods in smallholder farming systems, and in particular within the context of climate change effects is reviewed. Firstly, factors associated with citron watermelon adaptation to drought at morpho-physiological and molecular levels and how this relates to yield performance are revised with a systematic review approach. Secondly, citron watermelon's potential role in adding to crop diversification in the smallholder farming systems is presented. Thirdly, alternative food uses and potential by-products that can be developed from citron watermelon for small-scale processing and value-addition are discussed. Fourth, Sub-Saharan farmers' role as key actors in the conservation of citron watermelon biodiversity is reviewed. Finally, the review summarizes significant findings and identifies critical knowledge gaps for further research.

2.2 Materials and methods

A systematic review approach was used to map the existing literature supporting the topic's broad research question. The systematic review methodology was based on the framework outlined by (Koutsos et al., 2019). The review included the following six steps: (i) scoping, (ii) planning, (iii) identification/searching, (iv) screening, (v) eligibility/assessment and (vi) presentation/interpretation (Figure 2.1).

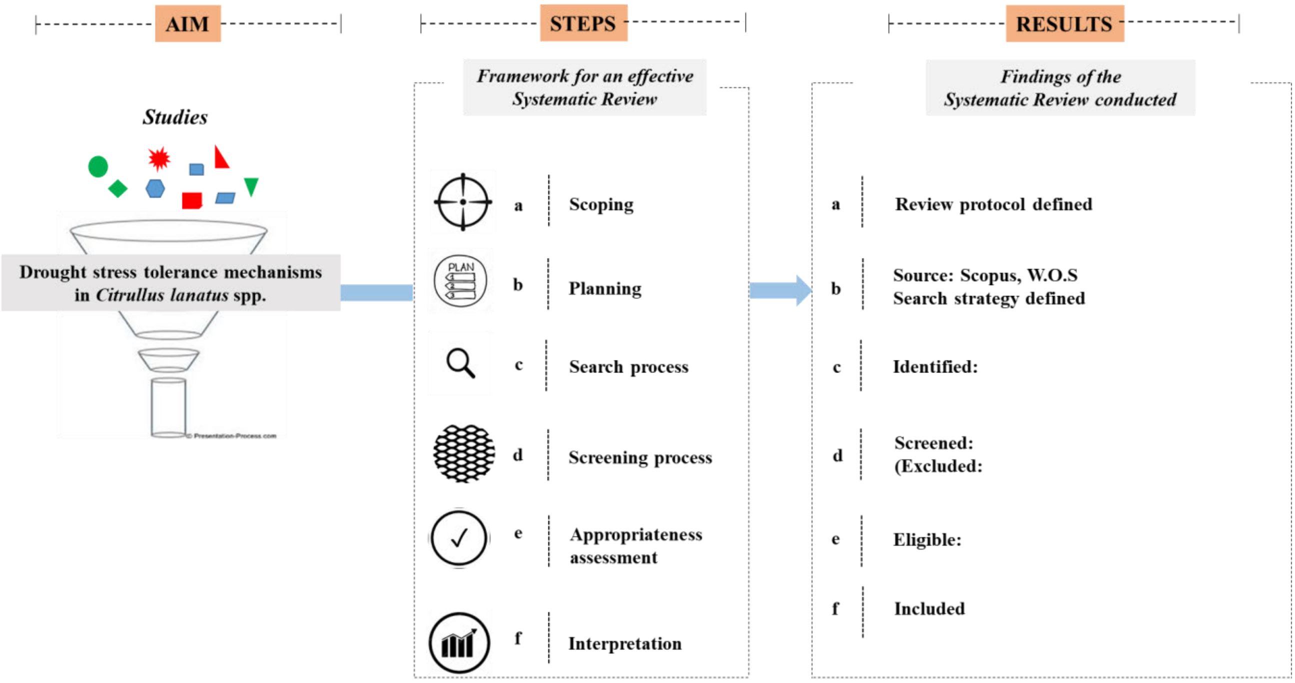

Figure 2.1: The framework used to perform a systematic review for current drought stress tolerance mechanisms in *C. lanatus* spp. [Adopted: (Koutsos et al., 2019)]

2.2.1 Research question

This review was guided by the question, “What is the current understanding of drought stress tolerance mechanisms in *Citrullus lanatus* spp.? Citron watermelon is a C3 crop that is known to be less photo-efficient. It is worthy of understanding how this species deal with the problem of Rubisco having an affinity for oxygen at low CO_2 concentrations.

2.2.2 Data sources and search strategy

The search was implemented in five electronic databases; (i) Scopus (*www.scopus.com*), (ii) Web of Science (*www.webofknowledge.com*) (iii) Science Direct (*www.sciencedirect.com*), (iv) Science.gov (*www.science.gov*) and (v) Google Scholar (*scholar.google.com*). These databases were selected to be inclusive and cover disciplines in agriculture sciences. Limits on database search included peer-reviewed literature published from 1 January 1995 to 31 December 2019. The date range limitation was chosen to focus on contemporary literature on drought tolerance mechanisms. The search strategy employed broad search terms (Table 2.1) to ensure publications were not overlooked.

Table 2.1: Search strategy with Boolean operators for each database to identify peer-reviewed articles examining drought stress tolerance mechanisms in *C. lanatus* spp.

Database(s)	Primary term(s)	Expanded term(s)
Scopus Web of Science Science Direct Science.gov Google Scholar	Drought stress	“water stress” **OR** “moisture stress” **OR** “water deficit” **OR** “water shortage” **OR** “water scarcity” **AND**
	C. lanatus spp.	“watermelon” **OR** “citron watermelon” **OR** “desert watermelon” **OR** “wild watermelon” **OR** “melon” **OR** “muskmelon” **AND**
	Mechanism(s)	“adaptation strategy” **OR** “avoidance” **OR** “escape” **OR** “tolerance”

2.2.3 Citation management

Citations were imported into the DistillerSR (Evidence Partners Incorporated, Ottawa, ON, Canada) web-based application, and duplicate citations were removed using the duplicate removal function of DistillerSR. Subsequently, the title and abstract relevance screening and data characterisation of complete articles were carried out using DistillerSR.

2.2.4 Relevance screening and eligibility criteria

A two-step screening relevance technique was employed. For the first step of screening, the titles and abstracts of the articles were examined for relevance. Next, all citations considered relevant after the title and abstract screening went through a full-text review. Studies were eligible for inclusion if they were original articles on citron watermelon or *C. lanatus* spp. relevant to drought stress tolerance.

2.2.5 Data charting

The data collection categories included: author, year of publication, drought adaptation strategy, and key results. The data were compiled in a spreadsheet using the DistillerSR report function and subsequently imported into Microsoft Excel 2016.

2.2.6 Summarizing and reporting

A narrative synthesis approach was used to provide an overview of the existing literature. Firstly, a summary of the study findings was combined, considering the variations that may affect the generalization of drought tolerance mechanisms. Then, study results were organized into categories (drought avoidance, drought tolerance, and drought escape) using thematic analysis techniques (Saha et al., 2019).

2.3 Results

2.3.1 Overview of studies identified

The review resulted in three main themes explaining drought adaptation mechanisms in *C. lanatus* spp. The three main mechanisms are drought avoidance (DA), drought tolerance (DT), and drought escape (DE). Table 2.2 summarize 62 mechanisms from 52 articles from a systematic review on drought adaptation mechanisms conducted in the past 25 years (1995-2019). The drought tolerance (DT) mechanism had the highest number of articles (74%), followed by (DA) 21% and (DE) 5% (Figure 2.2).

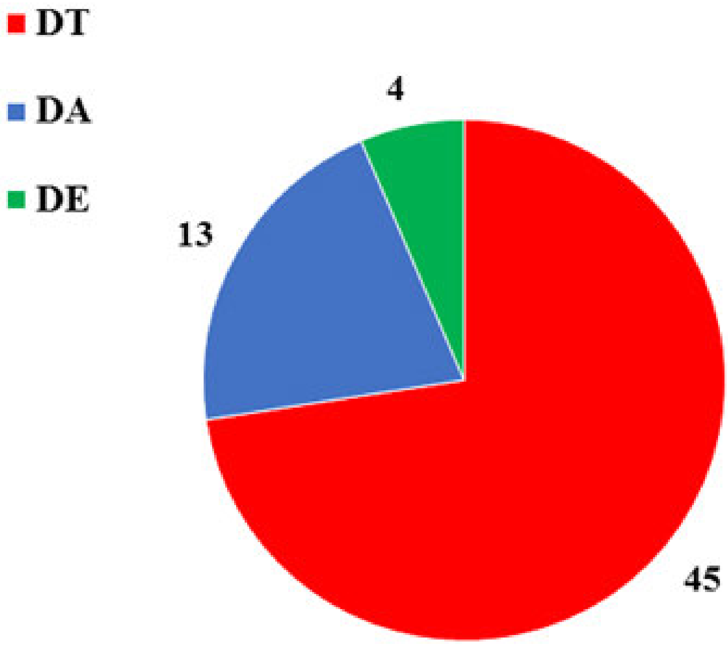

Figure 2.2: Pie chart summarizing 62 drought tolerance mechanisms in *C. lanatus* spp. from 62 articles

In the past 25 years, scientists have investigated the morphology, genetic and molecular mechanisms of drought response to enhance the drought tolerance in *Citrullus lanatus* spp. Drought tolerance mechanisms (45 articles) were more often investigated rather than DA (13 articles) and DE (4 articles). Drought tolerance studies in *Citrullus lanatus* spp. included change in gene expression (Akashi et al., 2004b; Baloglu et al., 2015; Celik Altunoglu et al., 2017; Hong et al., 2017; Kim et al., 2018; Li et al., 2012; Ram et al., 2015; Si et al., 2010; Unel et al., 2019; Wei et al., 2019; Yu et al., 2017) and accumulation of osmolytes (citrulline, glutamate, arginine) in leaves and roots (Sharma et al., 2019; Takahara et al., 2005; Takahara et al., 2006; Xu et al., 2018; Yang et al., 2017; Yokota et al., 2002; Yoshimura et al., 2008; Zhang et al., 2017). Drought avoidance studies include reduced leaf water loss (Akashi et al., 2016; D'Alessandro et al., 2019; Hakki et al., 2016; Kohzuma et al., 2007), enhanced water uptake (Kajikawa et al., 2010; Liu and Latimer, 1995; Omirou et al., 2013; Park et al., 2014), and accelerated transition from vegetative growth to reproductive growth to avoid complete abortion at the severe drought stress stage (Botha and Small, 1985; Kohzuma et al., 2007).

Table 2.2: Summary of studies on drought adaptation strategies in *C. lanatus* spp. [drought avoidance (DA), drought tolerance (DT), drought escape (DE)]

			Mechanisms		
Author	**Title**	**Research summary**	**DA**	**DT**	**DE**
(Akashi et al., 2004a)	Expressed sequence tag-based gene expression analysis under drought stress in wild watermelon	Changes in gene expression in roots within 6 h water stress. Genes involved in oxidative stress (glutathione peroxidase, glucose-6-phosphate-dehydrogenase, and ascorbate peroxidase) were demonstrated to be regulated by water stress.		✓	
(Akashi et al., 2003)	Analysis of drought-induced metallothionein in wild watermelon	Gene (*CLMT2*) of the same homology with type-2 metallothionein contributed to the survival of wild watermelon under severe drought		✓	
(Akashi et al., 2005)	Agrobacterium-mediated transformation system for the drought and excess light stress-tolerant wild watermelon (*Citrullus lanatus*)	Experimental basis for molecular studies of wild watermelon genes helps understand their contribution to stress tolerance in this plant.		✓	
(Akashi et al., 2004b)	Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon	Gene (*CLMT2*) of same homology with type-2 metallothionein contributed to the survival of wild watermelon under severe drought		✓	
(Akashi et al., 2002)	Functional analysis of DRIP-1, a drought-induced polypeptide in wild watermelon	Wild watermelon accumulates high concentrations of citrulline, glutamate, and arginine in its leaves during drought		✓	
(Akashi et al., 2011)	Dynamic changes in the leaf proteome of a C_3 xerophyte, *Citrullus lanatus* (wild watermelon), in response to water deficit	Defense response of wild watermelon involves orchestrated regulation of functional proteins, of which HSPs play a pivotal role in the protection of the plant under water deficit		✓	
(Akashi and Yokota, 2003)	Molecular responses of wild watermelon to drought stress	Rapid accumulation of HSPs in stressed melons		✓	
(Akashi et al., 2016)	Potential involvement of drought-induced Ran GTPase CLRan1 in root growth enhancement in a xerophyte wild watermelon	Ran GTPase (CLRan1), expressed in the roots of drought-resistant wild watermelon, functions as a positive factor for maintaining root growth under osmotic stress.	✓	✓	
(Ansari et al., 2019)	Drought mediated physiological and molecular changes in muskmelon (*Cucumis melo* L.)	Increased activity of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and guaiacol (POD). Under drought stress, muskmelon elevates the abundance of defense proteins and suppresses catabolic proteins	✓	✓	
(Baloglu et al., 2015)	Identification, molecular characterization and expression analysis of RPL24 genes in three Cucurbitaceae family members: cucumber, melon and watermelon	Ribosomal protein L24 (RPL24) is responsible for stabilizing the peptidyl transferase activity—increased expression of CmRPL24-01 genes in melon leaf tissue at 3 h upon polyethylene glycol treatment.		✓	
(Baragé et al., 2006)	Identifying sources of water stress tolerance from wild species of the family *Cucurbitaceae* in vitro culture	Evaluating water stress tolerance capacity using the calus recuperation after dehydration under a laminar flux hot until a loss of 50% of their fresh weight as a basis.			✓
(Celik Altunoglu et al., 2017)	Genome-wide identification and comparative expression analysis of LEA genes in watermelon and melon genomes	Induction of LEA genes in root and leaf tissues after drought application		✓	
(Cheng et al., 2015)	Foliar Application of Abscisic Acid and Sulfonamide Compounds Induced Drought Tolerance in Watermelon	Sulfacetamide and Sulfasalazine improve drought resistance like ABA by increasing proline, glycine betaine and malondialdehyde, and ascorbate peroxidase activity.		✓	
(D'Alessandro et al., 2019)	The Apocarotenoid beta-Cyclocitric Acid Elicits Drought Tolerance in Plants	Volatile compound β-Cyclocitral (β-CC) in plant leaves, when converted to β-cyclocitric acid (β -CCA) it signals drought tolerance	✓	✓	
(Ekbic et al., 2017)	Assessment of watermelon accessions for salt tolerance using stress tolerance indices	GMP and STI indices indicated that G04 G14 and G21 could be prominent sources to develop drought tolerance.			✓
(Botha and Small, 1985)	Effect of water stress on the carbohydrate metabolism of *Citrullus lanatus* seeds during germination	Stressed seeds lower the rate of $^{14}CO_2$ release from [2-^{14}C]acetate, [1-^{14}C]glucose, and [6-^{14}C]glucose	✓	✓	
(Hakki et al., 2016)	Physiological responses of two contrasting watermelon genotypes exposed to drought and nitric oxide	Drought stress decreased fresh and dry weights of shoots and roots and lengths drought-sensitive genotype KAR 147	✓		
(Hamurcu et al., 2016)	Biochemical effects of drought stress on two Turkish watermelon varieties are different and influenced by nitric oxide	MDA (a marker of oxidative damage on lipid membranes) was increased due to drought in watermelon genotypes and NO treatment slightly reduced MDA contents under drought stress.		✓	
(Hong et al., 2017)	Expression Analysis of Five Arabidopsis PDLP5 Homologous in Watermelon Subjected to Biotic and Abiotic Stresses	Plasmodesmata-located protein 5 (PDLP5) controls cell-to-cell communication and defense signalling		✓	

(Kajikawa et al., 2010)	Establishment of a transgenic hairy root system in wild and domesticated watermelon (*Citrullus lanatus*) for studying root vigour under drought	A powerful tool for the comparative study of the molecular mechanism underlying drought-induced root growth in desert plants	✓	✓	
(Kim et al., 2015)	Watermelon (*Citrullus lanatus*) late-embryogenesis abundant group 3 protein, ClLEA3-1, responds to diverse abiotic stresses	A representative group of LEA proteins ClLEA3-1 (Cl017745) can be used as an abiotic stress marker gene in watermelon		✓	
(Kim et al., 2018)	Abiotic stress and tissue-specific reference genes for quantitative reverse transcription PCR analysis in Korean native watermelons, *Citrullus lanatus* 'Black-King' and 'Speed-Plus-Honey'	Reference genes (ClACT and ClEF1α) were expressed in flowers, leaves, tendrils, stem, and roots after drought treatment		✓	
(Kohzuma et al., 2007)	Preferential decay of the CF1 epsilon subunit induces thylakoid uncoupling in wild watermelon under drought stress	Selective decomposition of epsilon subunit induces uncoupling of thylakoid membranes under drought and contributes to the avoidance of over-acidification in the thylakoid lumen under excess light conditions.	✓		
(Li et al., 2012)	Expression analysis of beta-glucosidase genes that regulate abscisic acid homeostasis during watermelon (*Citrullus lanatus*) development and under stress conditions	Beta-glucosidase genes regulate ABA content during drought stress		✓	
(Liu and Latimer, 1995)	Water relations and Abscisic-Acid levels of watermelon as affected by rooting volume restriction	ABA act as a signal for reduced growth of plants under Rooting volume restriction (RVR) conditions.	✓	✓	
(Malambane et al., 2018)	Comparative effects of ethylene inhibitors on Agrobacterium-mediated transformation of drought-tolerant wild watermelon	Controlling ethylene level during co-cultivation and shoot formation using the *cad's-harbour*ing Agrobacterium enhances drought tolerance		✓	
(Masuda et al., 2006)	Proteomic analysis of avoidance and defence mechanisms to drought stress in the root of wild watermelon	Defense response of wild melons involves orchestrated regulation of functional proteins	✓	✓	
(Nanasato et al., 2005)	Co-expression of cytochrome b_{561} and ascorbate oxidase in leaves of wild watermelon under drought and high light conditions	Levels of cDNA (CLb561A) mRNA and protein were elevated in the leaves during drought		✓	
(Nawaz et al., 2018)	Genome-wide expression profiling of leaves and roots of watermelon in response to low nitrogen	Under abiotic stress, leaf tissues are more sensitive compared with root tissues. 9598 genes were differentially expressed, out of which 4533 genes were up-regulated, and 5065 genes were down-regulated		✓	
(Omirou et al., 2013)	Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots but leads to improved colonization and plant response under water stress	Plant inoculation with mycorrhizal fungi was related to the response of plants to water stress conditions by improving WUE	✓		
(Park et al., 2014)	Ectopic expression of Arabidopsis H^+-pyrophosphatase AVP1 enhances drought resistance in bottle gourd (*Lagenaria siceraria* Standl.)	Wild-type plants showed minimal growth while the *AVP*-1 expressing plants resumed rapid growth, displaying longer ramified primary roots	✓		
(Ram et al., 2015)	Molecular cloning and in silico analysis of DREB-like gene in watermelon	DREB genes were identified from watermelon related to drought-tolerant genes.		✓	
(Ramirez et al., 2014)	Factors Affecting Germination of Citron melon (*Citrullus lanatus* var. *citroides*)	Citron melon can grow in a wide range of climatic conditions	✓	✓	
(Rouphael et al., 2017)	Vegetable grafting: a toolbox for securing yield stability under multiple stress conditions	Movement of mRNA through the phloem from rootstock to scion regulate plant growth and adaptation to drought stress			✓
(Sharma et al., 2019)	Phytohormones regulate accumulation of osmolytes under abiotic stress	Osmolytes (proline, glycine-betaine, polyamines, and sugars) are accumulated to safeguard the cellular machinery. Phytohormones (abscisic acid, brassinosteroids, cytokinins, ethylene, jasmonates, and salicylic acid) modulates the accumulation of osmolytes		✓	
(Si et al., 2010)	Cloning and expression analysis of the Ccrboh gene encoding respiratory burst oxidase in *Citrullus colocynthis* and grafting onto *Citrullus lanatus* (watermelon)	Drought responsive gene *Ccrboh* is functionally important during the acclimation of plants to stress, and it's promising for improving drought tolerance of other cucurbit species		✓	
(Si et al., 2009)	Gene expression changes in response to drought stress in *Citrullus colocynthis*	During drought, stress-responsive genes and plant hormones are involved in an extensive cross-talk		✓	
(Takahara et al., 2005)	Biochemical and molecular characterization of glutamate N-acetyltransferase involved in citrulline accumulation in wild watermelon during drought/strong-light stresses	Exogenous acetylene triggers the accumulation of citrulline to maintain the plant membrane structure		✓	
(Takahara et al., 2006)	Regulation of metabolic pathways for the massive accumulation of citrulline during drought/strong light stress in wild watermelon	Rapid accumulation of citrulline in leaves and shoot under drought stress		✓	

(Unel et al., 2019)	Comparative identification, characterization, and expression analysis of bZIP gene family members in watermelon and melon genomes	Genes (ClabZIP and CmbZIP) were expressed in leaf and root tissues after the drought was imposed		✓	
(Wei et al., 2019)	Comparative analysis of Calcium-Dependent Protein Kinase in Cucurbitaceae and expression studies in watermelon	The study provides insights into the evolutionary history of gene families in Cucurbitaceae and indicates a subset of candidate genes for functional characterizations		✓	
(Xu et al., 2018)	Glycinebetaine biosynthesis in response to osmotic stress depends on jasmonate signalling in watermelon suspension cells	Osmotic stress-induced glycinebetaine biosynthesis occurs via JA signal transduction and contributes to osmotic stress hardening		✓	
(Yang et al., 2017)	Genome-Wide Identification and Expression Analysis of NF-Y Transcription Factor Families in Watermelon (*Citrullus lanatus*)	The study provides a foundation for further functional analysis of NF-Y proteins during watermelon development and responses to drought stress. The results will be valuable for evolutionary analysis of the NF-Y family in *Cucurbitaceae* species.		✓	
(Yang et al., 2018)	Identification and expression analyses of WRKY genes reveal their involvement in growth and abiotic stress response in watermelon (*Citrullus lanatus*)	A total of 63 putative WRKY genes in watermelon were reported to regulate respective target genes		✓	
(Yokota et al., 2002)	Citrulline and DRIP-1 protein (ArgE homologue) in drought tolerance of wild watermelon	Wild watermelon accumulates high concentrations of citrulline, glutamate, and arginine in its leaves during drought		✓	
(Yoshimura et al., 2008)	Programmed proteome response for drought avoidance/tolerance in the root of a C_3 xerophyte (wild watermelon) under water deficits	Defense response of wild watermelon involves orchestrated regulation of functional proteins, of which HSPs play a pivotal role in the protection of the plant under water deficit		✓	
(Yoshida et al., 2005)	Proteomic analysis of drought/strong light stress responses in wild watermelon leaves	DREB genes were identified from watermelon related to drought-tolerant genes.		✓	
(Yoshimura et al., 2005)	Identification of drought-responsible proteins in the root of wild watermelon by proteomic analysis	DREB genes were identified from watermelon related to drought-tolerant genes.		✓	
(Yoshimura et al., 2006)	Regulation of the root development mechanism involved in Ran GTPase of wild watermelon under drought stress	Ran GTPase genes function in watermelon development, as well as in response to abiotic stress and hormones		✓	
(Yu et al., 2017)	Genome-wide identification and expression analysis of ClLAX, ClPIN, and ClABCB genes families in Citrullus lanatus under various abiotic stresses and grafting	Expression genes (ClLAX, ClPIN, and ClABCB) under drought helps to understand the roles of auxin transporter genes in watermelon adaptation to environmental stresses		✓	
(Zhang et al., 2017)	Antioxidant enzymes activities in leaves and yield analysis of different ecological types watermelon under drought stress	The content of proline, the activity of SOD, POD, CAT was genotype related	✓	✓	✓
(Zhou et al., 2018)	Identification and characterization of the glutathione peroxidase (GPX) gene family in watermelon and its expression under various abiotic stresses	ClGPX genes function in watermelon development, as well as in response to abiotic stress and hormones		✓	

2.4 Discussion

In response to water deficits or drought stress conditions, desert xerophytes have evolved a series of mechanisms at morphological, physiological and molecular levels to proceed with normal plant function and metabolism. These mechanisms include drought escape (DE) through early completion of a plant life cycle, drought avoidance (DA) through improved water absorbance capacity by the improved root system, and shedding of leaves (Figure 2.3). Drought tolerance (DT) occurs through alteration of the metabolic pathway (for example, increased antioxidant metabolism).

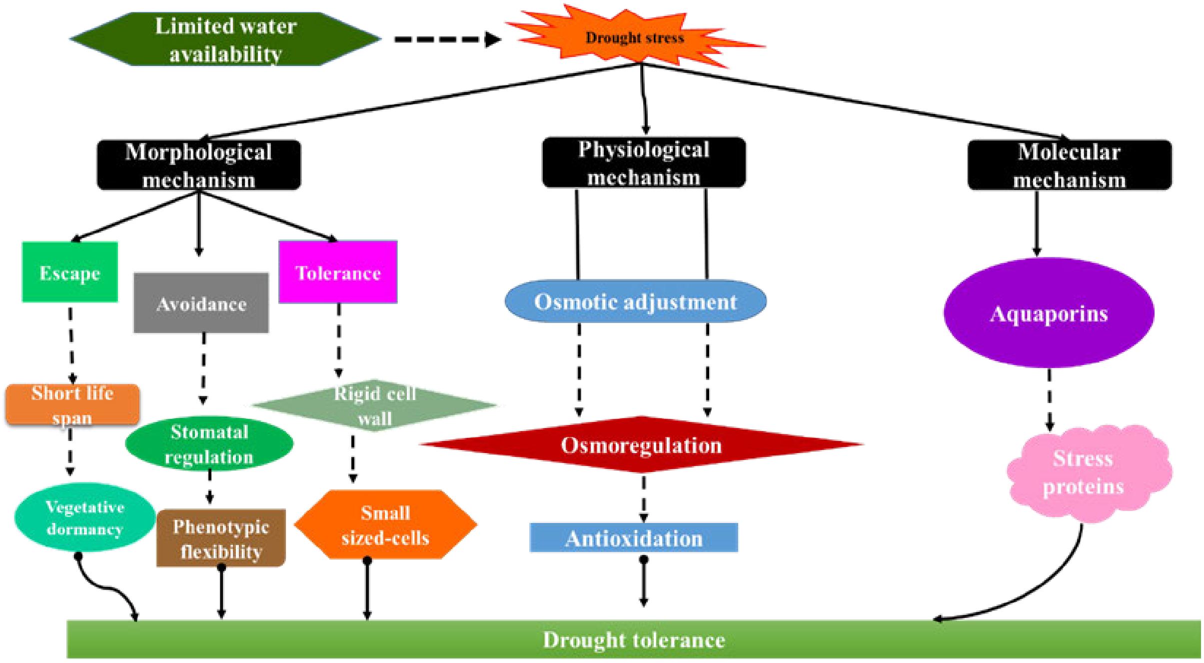

Figure 2.3: Plants adopting morphological, physiological, and molecular behaviour under drought stress [Author's drawing]

Citrullus lanatus spp. can minimize water loss through transpiration by maintaining basic physiological processes under drought stress conditions, that is, adjusting morphological features (hairy leaf surface and shedding leaves) (Blum, 2005; Luo, 2010). Primarily DA is characterized by maintaining high plant water potentials under water stress conditions (Mitra, 2001). *Citrullus. lanatus* spp. has been reported to respond to drought using the following DA mechanisms: (i) reducing water loss by partial closure of stomatal pores, leaf rolling (Tardieu, 2013), and wax accumulation on the leaf surface (Cameron et al., 2006; Islam et al., 2009); (ii) enhancing water uptake ability through a well-developed ramified root system. (Park et al., 2014), and (iii) accelerating/decelerating the transition from vegetative growth to reproductive growth to avoid complete abortion under severe drought stress (Kohzuma et al., 2007).

Citron watermelon escapes drought by adjusting its growth period (Franks, 2011). Plant phenology has a decisive effect on yield under water stress conditions. Early maturity in citron watermelon (Akashi et al., 2016) correlates with root length density to leaf area ratio, which translates to the plant's ability to maintain high leaf water potential under soil moisture stress. Plants that escape drought, such as the desert ephemeral (*Alyssum alyssoides* L.), exhibit early flowering, short plant life cycle, and developmental plasticity (Sherrard and Maherali, 2006). Citron watermelon was reported to escape drought through early flowering rather than avoid drought through increased water-use efficiency (Yoshimura et al., 2005). While a short growth period is correlated with reduced yield potential, if the specified cultivar's target environment represents a definitive stress area, then the early genotype gain under stress outweighs its potential yield deficiency.

From the systematic review results, it is evident that our understanding of the molecular mechanisms underlying drought tolerance in *C. lanatus* spp. is limited to the accumulation of abscisic acid (ABA) and heat shock proteins (HSPs). Literature has not explained phytosterols' role (a group of hormones that are essential for regulating plant development and morphogenesis) under drought stress in citron watermelon. Future genome-scale studies involving stress signalling pathways in *C. lanatus* spp. are necessary, given that drought response is dependent on species and genotype. Data obtained from such experiments can be applied to build network models, establishing a link between phenotypic traits with regulatory mechanisms. In addition to this, current and future generation DNA sequencing technology, high throughput phenotyping platforms, and improved informatics resources, expediting gene discovery will be key for improving abiotic stress tolerance in this crop.

Citron watermelon, a C3 xerophyte, can survive absolute moisture stress (Akashi et al., 2008; Gorai et al., 2015; Hsiao and Acevedo, 1975). Despite its potential utility as a source of genes for drought tolerance breeding, functional genomics of *C. lanatus* spp. has been limited by the lack of genetic approaches and complexity of the phenomenon, and there is a need to bridge this gap. However, with the realization of "omics" technologies, it is possible to provide a comprehensive description of changes in the transcript, proteome, and metabolome levels during drought stress. Combining the data on phenomics and genomics should lead to a systems biology approach and identify target genes and critical metabolic pathways. This process's complete elucidation would enable interpreting the incredible nature of C3 xerophytes.

2.4.1 Citron watermelon contribution to human nutrition and health

Citron watermelon could potentially fulfill nutritional requirements by supplying biochemical compounds with health-promoting properties. In watermelon seeds (same family with citron watermelon), four proteinogenic amino acids (phenylalanine, threonine, tryptophan, and valine) have been identified, and proteins are accumulated in quantities higher than those found in cereals (10-15%). Watermelon seeds also contain vitamins A, C, D, E, and K and several antioxidants such as flavonoids (Guo et al., 2003). Populations consuming flavonoids-enriched foods have low cancer frequency (Paliyath et al., 2011). Also, lactating mothers eating citron watermelon seeds can produce higher quality milk, as seen in animal models fed with isoflavone-rich fodder (Zhengkang et al., 2006). Flavonoids can inhibit degenerative diseases like coronary heart disease, atherosclerosis, cancer, diabetes, and Alzheimer's disease through their antioxidant activity and modulating multiple protein functions (Pandey and Rizvi, 2009). Also, the absence of gluten in citron watermelon seeds offers alternative nourishment for the celiac population (people with a reaction to eating gluten), and it could counteract the increasing problems of obesity in the developed world. In less developed countries, citron watermelon could significantly reduce malnutrition and death by hunger.

2.4.2 The preservation of citron watermelon biodiversity

Citron watermelon seeds of different accessions are currently being conserved in several seed banks worldwide (*ex-situ* conservation). However, preserving agrobiodiversity means preserving indigenous farmers' associated culture living in the Sub-Saharan Africa region (Jarvis et al., 2000). The importance of seed banks in the conservation of biodiversity is well known, and the success of future conservation and breeding programs hinges on the preservation of this diversity on-farm. Moreover, the transfer of indigenous knowledge and associated practices will help adapt citron watermelon to new regions. Citron watermelon is a crop of family heritage; knowledge is acquired from the parents who have cultivated the crop since childhood (Swislocki, 2008). Mujaju et al. (2018) pointed out that farmers in Sub-Saharan Africa are key role players in the preservation of genetic diversity of citron watermelon in their fields, and they have the expertise for the agronomic management of their accessions.

Industrial development is causing migration from rural areas to the cities (Zadawa and Omran, 2020). In addition to the increasing demand for daily calories, this social and economic situation changes land use and increases the crop's genetic homogeneity. Due to better profits from staple crop exports and higher incomes from commercial farmers, small farmers migrate, putting their cultural and agro-biodiversity heritage at risk (Henriksson Malinga et al., 2018).

Therefore, it is of primary importance to preserve small-scale farming where the greatest genetic diversity of citron watermelon and associated human culture is found (Dwivedi et al., 2019). In Southern Africa, citron watermelon is still grown in the major historical areas of cultivation “the province of Limpopo, the province of Matabeleland, the province of Manicaland and Omaheke region,” an integral part of rural cultural heritage and identity (Mcgregor, 2012; Paris, 2016). Citron watermelon is a promising crop in a broader context. However, scientists and stakeholders must do all they can to preserve the heritage of citron watermelon so that this crop can continue to be cultivated while contributing to food quality and security in the SSA and globally.

2.5 Future research perspectives

Building on the literature examined, we identify six priority areas for research (summarized in Figure 2.4) and make recommendations for the short-and long-term development of citron watermelon as a crop that could contribute to food security and changing climatic conditions.

2.5.1 Synchronization of research and methods

According to the Global Biodiversity Information Facility (GBIF) (*https://www.gbif.org/*) (GBIF, 2019a; GBIF, 2019b), citron watermelon research and germplasm record include researchers from at least ten institutions in 13 countries. Despite positive national and international collaborations (Sultana and Ashraf, 2019), citron watermelon research is still disconnected with interesting and relevant research programs running in isolation (Mashilo et al., 2017; Ngwepe et al., 2019). This review aims to draw together many disparate aspects of citron watermelon research to facilitate researchers' body knowledge and collaboration. Also, we relate the experience of the BamNetwork (*http://bambaragroundnut.org/*) (CFF, 2018), the online representation of the international research community on Bambara groundnut (*Vigna subterranea* L.), which sought to bring together the expertise and enable close collaboration, the sharing of materials, resources, data, and technology. It is our view that citron watermelon research and food security in SSA could benefit from such an approach, with equitable and appropriate access and benefit-sharing agreements in place. Here, the suggestion is to develop a web database, which will act as an open repository for data emerging from citron watermelon research programs.

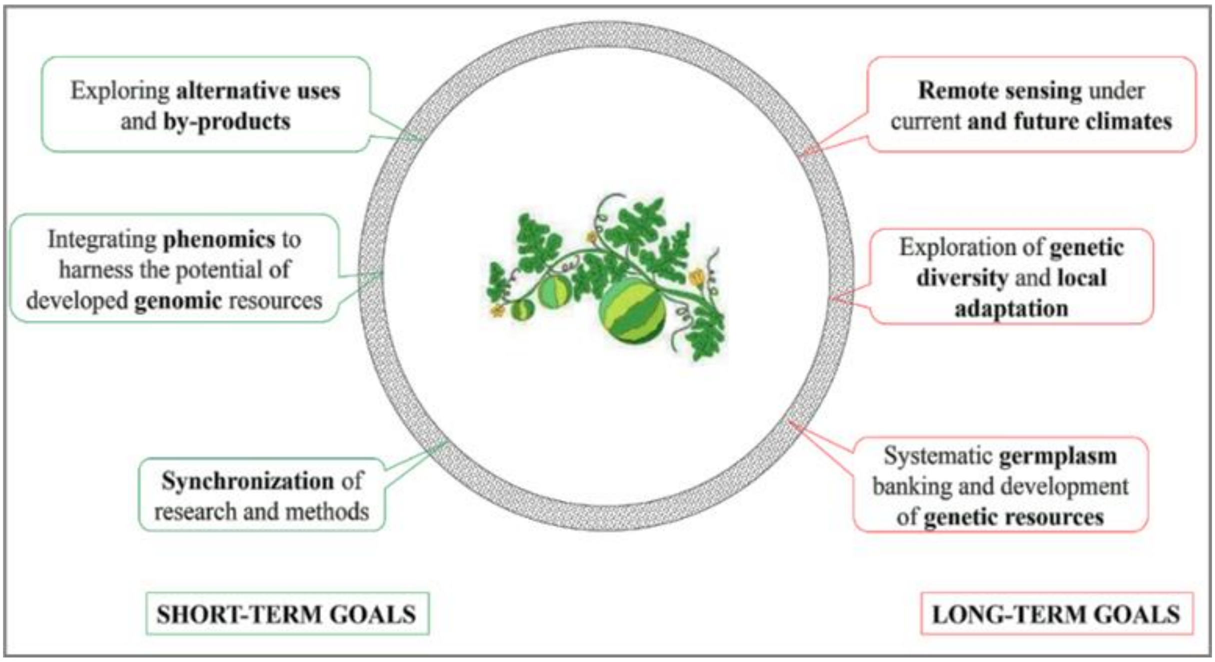

Figure 2.4: Roadmap for the sustainable development and exploitation of citron watermelon for food security and to support livelihoods [Author's drawing]

2.5.2 Integrating phenomics to harness the potential of developed genomic resources

The most productive farming land is facing biotic and abiotic stresses (fungal and bacterial diseases, heat, salinity, and drought stresses) (Godfray et al., 2010). All these biotic and abiotic stresses exert tremendous survival pressure on crops. Under the prevailing conditions and available resources, new plant varieties with desired traits (drought tolerance) and high yield potential need to be developed. This can be achieved by understanding the genetic makeup of plants (genomics) and their phenotype (phenomics) and the interaction between them in different environments.

In this era of phenomics, high-throughput precise phenotyping helps to amass high-quality, accurate phenotyping data. The high-quality phenotypic data is useful for meaningful genetic dissection and genomics assisted breeding for drought stress tolerance. The earlier use of destructive plant phenotyping methods now gives way to high-throughput non-destructive, precise imaging techniques. Several phenomics platforms (APPF, 2020) are now available with facilities allowing scientists new windows into living plants' inner machinery (Arend et al., 2016). These facilities embrace (i) infrared cameras to scan temperature profiles and transpiration, (ii) incandescent microscopy to measure photosynthesis, (iii) 3D camera to record precise changes in growth responses after crop plants are exposed to stresses, (iv) lidars (light detection) to measure growth rates, and (v) magnetic resonance imaging (MRI) to examine root and leaf physiology.

2.5.3 Exploring alternative uses

Citron watermelon has the potential to produce other valuable by-products. The leaves can be cooked as vegetables, and seeds can be roasted as snacks, ground into a powder, and used as a condiment. Exploring alternative uses requires indigenous knowledge from the local farmers where the crop is grown through conducting ethnobotanical surveys. In India, high-value protein and oils are extracted from a watermelon known as Mateera Beej (Mahla et al., 2014). The seeds contain 35-50% crude protein, 28-40% oil and minerals in significant quantities. Further, the oil contains more than 80% unsaturated fatty acids, with linoleic acid being the dominant fatty acid (68.3%). In citron watermelon, the chemical basis and nutrient composition have not been explored. Alka et al. (Alka et al., 2018) reported pharmacological activities (treatment of urinary tract infection, bed wetting, dropsy, and renal stones) of *Citrullus lanatus*.

2.5.4 Remote sensing under current and future climates

Estimates of the land area under citron watermelon cultivation (Zabel et al., 2014) and associated yields are highly variable. They have been hampered by poor record-keeping and difficult access to remote areas. The short-term nature of citron watermelon cultivation, local differences in cultivated landraces, plant growth rates, agronomic practices, and dependency on co-staple crop productivity in any given period make estimating citron watermelon production difficult. Therefore, standardized empirical analyses for both land areas suitable for citron watermelon cultivation and area currently under cultivation, yield components, and inter-annual trends are lacking.

Improvements in the resolution and accessibility of satellite data from National Aeronautics and Space Administration (NASA) products such as Moderate Resolution Imaging Spectroradiometer (MODIS) and Sentinel 2 are increasingly applied to vegetation and crop surveys (Alcantara et al., 2013; Ujoh et al., 2019). Therefore, there may be a potential to use freely accessible satellite data to monitor citron watermelon production directly in the near term. Furthermore, this approach could be applied to mapping the crop suitability of citron watermelon. Concomitantly, upgraded regional bioclimatic datasets (Worldclim2) and an improved network of climate stations and data loggers will allow better characterization of the citron watermelon environmental niche and stress conditions. The impact of climate change under a range of future scenarios is yet to be quantified for citron watermelon and will form an essential part of any future development strategy.

2.5.5 Exploration of genetic diversity and local adaptation

Citron watermelon genetic diversity distributed across different environmental conditions indicates that the process of domestication might have facilitated the adaptation of landraces to local conditions, and indeed to a wider range of conditions than its wild progenitor. Since citron watermelon is propagated by seed, this represents a robust system to investigate the genomic basis of drought adaptive traits. Key steps to achieve this would be the characterization of existing citron watermelon genetic diversity using high-resolution genomic markers, standardized methods to measure fitness and yield, and robust environmental conditions monitoring. Concurrently, assessing the risk of erosion of citron watermelon genetic diversity through the loss or decline of landraces should be a priority for future citron watermelon monitoring strategies. In the medium term, this could similarly be extended to monitoring of crop wild relative diversity. In the long term, with the prerequisite knowledge of germination biology, novel sexual breeding using mapping populations and pan-genomic sequencing may enable the development of improved genotypes, tolerant of diseases, better adapted to current and future climates, with desirable yield and by-product attributes.

2.5.6 Systematic germplasm banking and development of genetic resources

Citrullus lanatus species, particularly citron watermelon landraces, are currently severely underrepresented (Mcgregor, 2012). This chronically reduces the potential for plant breeding and crop improvement. In the long term, under scenarios of habitat loss, agricultural intensification, disease spread, climate change, and introduction of high-yielding genotypes, citron watermelon as an invaluable plant genetic resource is at risk of losing genetic diversity and consequently leading to the loss of genetic diversity.

While many landraces are present among subsistence farmers in Sub-Saharan Africa, citron watermelon germplasm management is vulnerable to outcrossing and poor documentation and needs commitment to proper maintenance(Mashilo et al., 2017). Therefore, further exploration of germplasm banking's potential from a wide range of spatial and environmental conditions is a crucial research objective. Conventional breeding and *ex-situ* seed conservation also require an understanding of desiccation, longevity of storage, and, essentially, the germination requirements. As with citron watermelon, much of this is not well understood. With appropriate access and benefit-sharing agreements, germplasm could be incorporated into established seed banks, benefiting research and sustainable exploitation and safeguarding an essential tropical crop.

In Nepal, a digital information system is currently under development as part of the Nepal Seed and Fertilizer (NSAF) project, funded by the United States Agency for International Development (USAID) (CGIAR_Big_Data, 2020). This system allows easy access to an electronic seed catalogue with all registered varieties' features and sources; simultaneously, the balance sheet collects and shares information on seed demand and supply by all stakeholders in real-time.

2.6 Conclusion

Under the context of climate change and crop production, citron watermelon is an interesting plant species whose capacity to tolerate adverse environmental conditions (water stress) and remarkable nutritional qualities warrant further research in all fields of plant biology, agronomy, and ecology. We projected short term and long-term goals integrating the fundamental factors that explain and determine the future of citron watermelon regarding food security, biodiversity conservation, and crop diversification. Additionally, smallholder farmers should be encouraged to rely on a broader range of genotypes to sustain small-scale crop production and their economic, social, and cultural interactions. This will reinforce local conservation dynamics and ensure the sustainability of citron watermelon locally and around the world.

References

Akashi K., Hashizume T., Yamashita A., Toh H., Hattori M., Yokota A. (2004a) Expressed sequence tag-based gene expression analysis under drought stress in wild watermelon. Plant and Cell Physiology 45:S94-S94.

Akashi K., Ishida Y., Nishimura N., Yokota A. (2003) Analysis of drought-induced metallothionein in wild watermelon. Plant and Cell Physiology 44:S1-S1.

Akashi K., Miyake C., Yokota A. (2001) Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. Febs Letters 508:438-442. DOI: Doi 10.1016/S0014-5793(01)03123-4.

Akashi K., Morikawa K., Yokota A. (2005) Agrobacterium-mediated transformation system for the drought and excess light stress-tolerant wild watermelon (*Citrullus lanatus*). Plant Biotechnology 22:13-18. DOI: 10.5511/plantbiotechnology.22.13.

Akashi K., Nishimura N., Ishida Y., Yokota A. (2004b) Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon. Biochem Biophys Res Commun 323:72-8. DOI: 10.1016/j.bbrc.2004.08.056.

Akashi K., Takahara K., Yamada R., Miyake C., Yokota A. (2002) Functional analysis of DRIP-1, a drought-induced polypeptide in wild watermelon. Plant and Cell Physiology 43:S112-S112.

Akashi K., Yokota A. (2003) Molecular responses of wild watermelon to drought stress. Plant and Cell Physiology 44:S111-S111.

Akashi K., Yoshida K., Kuwano M., Kajikawa M., Yoshimura K., Hoshiyasu S., Inagaki N., Yokota A. (2011) Dynamic changes in the leaf proteome of a C_3 xerophyte, *Citrullus lanatus* (wild watermelon), in response to water deficit. Planta 233:947-60. DOI: 10.1007/s00425-010-1341-4.

Akashi K., Yoshimura K., Kajikawa M., Hanada K., Kosaka R., Kato A., Katoh A., Nanasato Y., Tsujimoto H., Yokota A. (2016) Potential involvement of drought-induced Ran GTPase CLRan1 in root growth enhancement in a xerophyte wild watermelon. Biosci Biotechnol Biochem 80:1907-16. DOI: 10.1080/09168451.2016.1191328.

Akashi K., Yoshimura K., Nanasato Y., Takahara K., Munekage Y., Yokota A. (2008) Wild plant resources for studying molecular mechanisms of drought/strong light stress tolerance. Plant Biotechnology 25:257-263. DOI: DOI 10.5511/plantbiotechnology.25.257.

Alam K., Rahman M.H. (2014) Women in natural disasters: A case study from southern coastal region of Bangladesh. International Journal of Disaster Risk Reduction 8:68-82. DOI: 10.1016/j.ijdrr.2014.01.003.

Alcantara C., Kuemmerle T., Baumann M., Bragina E.V., Griffiths P., Hostert P., Knorn J., Muller D., Prishchepov A.V., Schierhorn F., Sieber A., Radeloff V.C. (2013) Mapping the extent of abandoned farmland in Central and Eastern Europe using MODIS time series satellite data. Environmental Research Letters 8:035035. DOI: 10.1088/1748-9326/8/3/035035.

Alka G., Anamika S., Ranu P. (2018) A review on watermelon (*Citrullus lanatus*) medicinal seeds. Journal of Pharmacognosy and Phytochemistry 7:2222-2225.

Ansari W.A., Atri N., Ahmad J., Qureshi M.I., Singh B., Kumar R., Rai V., Pandey S. (2019) Drought mediated physiological and molecular changes in muskmelon (*Cucumis melo* L.). PLoS One 14:e0222647. DOI: 10.1371/journal.pone.0222647.

APPF. (2020) The Australian Plant Phenomics Facility, The Australian Plant Phenomics Facility, Australia.

Arend D., Junker A., Scholz U., Schuler D., Wylie J., Lange M. (2016) PGP repository: a plant phenomics and genomics data publication infrastructure. Database (Oxford) 2016. DOI: 10.1093/database/baw033.

Baloglu M.C., Ulu F., Altunoglu Y.C., Pekol S., Alagoz G., Ese O. (2015) Identification, molecular characterization and expression analysis of RPL24 genes in three Cucurbitaceae family members: cucumber, melon and watermelon. Biotechnology & Biotechnological Equipment 29:1024-1034. DOI: 10.1080/13102818.2015.1079144.

Baragé M., Balla A., Adam T. (2006) Identifying sources of water stress tolerance from wild species of the family *Cucurbitaceae* in vitro culture. Biotechnology, Agronomy and Society and Environment 10:3-6.

Blum A. (2005) Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research 56:1159-1168. DOI: 10.1071/Ar05069.

Botha F.C., Small J.G. (1985) Effect of Water Stress on the Carbohydrate Metabolism of Citrullus lanatus Seeds during Germination. Plant Physiol 77:79-82. DOI: 10.1104/pp.77.1.79.

Cameron K.D., Teece M.A., Smart L.B. (2006) Increased accumulation of cuticular wax and expression of lipid transfer protein in response to periodic drying events in leaves of tree tobacco. Plant Physiol 140:176-83. DOI: 10.1104/pp.105.069724.

Celik Altunoglu Y., Baloglu M.C., Baloglu P., Yer E.N., Kara S. (2017) Genome-wide identification and comparative expression analysis of LEA genes in watermelon and melon genomes. Physiol Mol Biol Plants 23:5-21. DOI: 10.1007/s12298-016-0405-8.

CFF. (2018) BamNetWork, Crops For the Future.

CGIAR_Big_Data. (2020) Seed systems in Nepal are going digital, in: Twitter (Ed.), Twitter. pp. Tweet.

Cheng W.S., Zeng D.L., Zhang N., Zeng H.X., Shi X.F., Li Y.H., Sun Y.H. (2015) Foliar Application of Abscisic Acid and Sulfonamide Compounds Induced Drought Tolerance in Watermelon. Applied Mechanics and Materials 723:705-710. DOI: 10.4028/www.scientific.net/AMM.723.705.

Chivenge P., Mabhaudhi T., Modi A.T., Mafongoya P. (2015) The Potential Role of Neglected and Underutilised Crop Species as Future Crops under Water Scarce Conditions in Sub-Saharan Africa. Int J Environ Res Public Health 12:5685-711. DOI: 10.3390/ijerph120605685.

Chomicki G., Schaefer H., Renner S.S. (2020) Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. New Phytol 226:1240-1255. DOI: 10.1111/nph.16015.

D'Alessandro S., Mizokami Y., Legeret B., Havaux M. (2019) The Apocarotenoid beta-Cyclocitric Acid Elicits Drought Tolerance in Plants. iScience 19:461-473. DOI: 10.1016/j.isci.2019.08.003.

Dwivedi S., Goldman I., Ortiz R. (2019) Pursuing the Potential of Heirloom Cultivars to Improve Adaptation, Nutritional, and Culinary Features of Food Crops. Agronomy-Basel 9:441. DOI: 10.3390/agronomy9080441.

Ekbic E., Cagiran C., Korkmaz K., Kose M.A., Aras V. (2017) Assessment of watermelon accessions for salt tolerance using stress tolerance indices. Ciencia E Agrotecnologia 41:616-625. DOI: 10.1590/1413-70542017416013017.

FAOSTAT. (2019) Crop Production Data, Rome, Italy.

Franks S.J. (2011) Plasticity and evolution in drought avoidance and escape in the annual plant *Brassica rapa*. New Phytol 190:249-57. DOI: 10.1111/j.1469-8137.2010.03603.x.

GBIF. (2019a) *Citrullus lanatus* var. *citroides* (L.H.Bailey) Mansf. in Wiersema J H. Kulturpflanze, Beih. 2:422. 1959. DOI: 10.15468/ao14pp.

GBIF. (2019b) Free and open access to biodiversity data, Global Biodiversity Information Facility, Denmark, Copenhagen.

Gil J.D.B., Reidsma P., Giller K., Todman L., Whitmore A., van Ittersum M. (2019) Sustainable development goal 2: Improved targets and indicators for agriculture and food security. Ambio 48:685-698. DOI: 10.1007/s13280-018-1101-4.

Godfray H.C., Beddington J.R., Crute I.R., Haddad L., Lawrence D., Muir J.F., Pretty J., Robinson S., Thomas S.M., Toulmin C. (2010) Food security: the challenge of feeding 9 billion people. Science 327:812-8. DOI: 10.1126/science.1185383.

Gorai M., Laajili W., Santiago L.S., Neffati M. (2015) Rapid recovery of photosynthesis and water relations following soil drying and re-watering is related to the adaptation of desert shrub Ephedra alata subsp alenda (Ephedraceae) to arid environments. Environmental and Experimental Botany 109:113-121. DOI: 10.1016/j.envexpbot.2014.08.011.

Guo C.J., Yang J.J., Wei J.Y., Li Y.F., Xu J., Jiang Y.G. (2003) Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutrition Research 23:1719-1726. DOI: 10.1016/j.nutres.2003.08.005.

Gusmini G., Wehner T.C., Jarret R.L. (2004) Inheritance of egusi seed type in watermelon. J Hered 95:268-70. DOI: 10.1093/jhered/esh031.

Hakki E.E., Hamurcu M., Gezgin S., Demiral T. (2016) Physiological responses of two contrasting watermelon genotypes exposed to drought and nitric oxide. Journal of Biotechnology 231:S25-S25. DOI: 10.1016/j.jbiotec.2016.05.110.

Hamurcu M., Hakki E.E., Demiral T., Gezgin S. (2016) Biochemical effects of drought stress on two Turkish watermelon varieties are different and influenced by nitric oxide. Journal of Biotechnology 231:S26-S26. DOI: 10.1016/j.jbiotec.2016.05.111.

Henriksson Malinga R., Jewitt G.P.W., Lindborg R., Andersson E., Gordon L.J. (2018) On the other side of the ditch: exploring contrasting ecosystem service coproduction between smallholder and commercial agriculture. Ecology and Society 23. DOI: 10.5751/es-10380-230409.

Hong J.P., Suh H.Y., Kim J.H., Ahn Y.K., Kim J., Lee E.S., Kim D.S., Lee H.E. (2017) Expression Analysis of Five Arabidopsis PDLP5 Homologous in Watermelon Subjected to Biotic and Abiotic Stresses. Horticulture Environment and Biotechnology 58:367-375. DOI: 10.1007/s13580-017-0137-x.

Hsiao T.C., Acevedo E. (1975) Plant responses to water deficits, water-use efficiency, and drought resistance, Developments in Agricultural and Managed Forest Ecology, Elsevier. pp. 59-84.

Islam M.A., Du H., Ning J., Ye H., Xiong L. (2009) Characterization of Glossy1-homologous genes in rice involved in leaf wax accumulation and drought resistance. Plant Mol Biol 70:443-56. DOI: 10.1007/s11103-009-9483-0.

Janick J., Paris H.S., Parrish D.C. (2007) The cucurbits of mediterranean antiquity: identification of taxa from ancient images and descriptions. Ann Bot 100:1441-57. DOI: 10.1093/aob/mcm242.

Jarvis D.I., Myer L., Klemick H. (2000) A training guide for in situ conservation on-farm Bioversity International.

Kajikawa M., Morikawa K., Abe Y., Yokota A., Akashi K. (2010) Establishment of a transgenic hairy root system in wild and domesticated watermelon (*Citrullus lanatus*) for studying root vigor under drought. Plant Cell Rep 29:771-8. DOI: 10.1007/s00299-010-0863-3.

Kim S.J., Huh Y.C., Ahn Y.K., Kim J., Kim D.S., Lee H.E. (2015) Watermelon (*Citrullus lanatus*) late-embryogenesis abundant group 3 protein, ClLEA3-1, responds to diverse abiotic stresses. Horticulture Environment and Biotechnology 56:555-560. DOI: 10.1007/s13580-015-0054-9.

Kim S.J., Jung I.L., Lee H.E., Lee J.H. (2018) Abiotic stress and tissue-specific reference genes for quantitative reverse transcription PCR analysis in Korean native watermelons, Citrullus lanatus 'Black-King' and 'Speed-Plus-Honey'. Applied Biological Chemistry 61:593-598. DOI: 10.1007/s13765-018-0391-3.

Kohzuma K., Akashi K., Munekage Y., Hisabori T., Yokota A. (2007) Preferential decay of the CF1 epsilon subunit induces thylakoid uncoupling in wild watermelon under drought stress. Photosynthesis Research 91:191-191. DOI: 10.1007/978-1-4020-6709-9_139.

Koutsos T.M., Menexes G.C., Dordas C.A. (2019) An efficient framework for conducting systematic literature reviews in agricultural sciences. Sci Total Environ 682:106-117. DOI: 10.1016/j.scitotenv.2019.04.354.

Levi A., Jarret R., Kousik S., Patrick Wechter W., Nimmakayala P., Reddy U.K. (2017) Genetic Resources of Watermelon, Genetics and Genomics of Cucurbitaceae, Springer. pp. 87-110.

Li Q., Li P., Sun L., Wang Y., Ji K., Sun Y., Dai S., Chen P., Duan C., Leng P. (2012) Expression analysis of beta-glucosidase genes that regulate abscisic acid homeostasis during watermelon (*Citrullus lanatus*) development and under stress conditions. J Plant Physiol 169:78-85. DOI: 10.1016/j.jplph.2011.08.005.

Liu A.M., Latimer J.G. (1995) Water Relations and Abscisic-Acid Levels of Watermelon as Affected by Rooting Volume Restriction. Journal of Experimental Botany 46:1011-1015. DOI: DOI 10.1093/jxb/46.8.1011.

Luo L.J. (2010) Breeding for water-saving and drought-resistance rice (WDR) in China. J Exp Bot 61:3509-17. DOI: 10.1093/jxb/erq185.

Mabhaudhi T., Chimonyo V.G.P., Hlahla S., Massawe F., Mayes S., Nhamo L., Modi A.T. (2019) Prospects of orphan crops in climate change. Planta 250:695-708. DOI: 10.1007/s00425-019-03129-y.

Mahla H.R., Singh J.P., Roy M.M. (2014) Seed purpose watermelon in arid zone Citeseer.

Malambane G., Nonaka S., Shiba H., Ezura H., Tsujimoto H., Akashi K. (2018) Comparative effects of ethylene inhibitors on Agrobacterium-mediated transformation of drought-tolerant wild watermelon. Biosci Biotechnol Biochem 82:433-441. DOI: 10.1080/09168451.2018.1431516.

Mashilo J., Shimelis H., Odindo A.O., Amelework B. (2017) Genetic diversity and differentiation in citron watermelon [*Citrullus lanatus* var. *citroides*] landraces assessed by simple sequence repeat markers. Scientia Horticulturae 214:99-106. DOI: 10.1016/j.scienta.2016.11.015.

Masuda A., Yoshimura K., Akashi K., Yokota A. (2006) Proteomic analysis of avoidance and defense mechanisms to drought stress in the root of wild watermelon. Plant and Cell Physiology 47:S101-S101.

Mcgregor C. (2012) *Citrullus lanatus* germplasm of southern Africa. Israel Journal of Plant Sciences 60:403-413. DOI: 10.1560/Ijps.60.1.403.

Mitra J. (2001) Genetics and genetic improvement of drought resistance in crop plants. Current Science 80:758-763.

Mujaju C., Zborowska A., Werlemark G., Garkava-Gustavsson L., Andersen S.B., Nybom H. (2011) Genetic diversity among and within watermelon (*Citrullus lanatus*) landraces in Southern Africa. Journal of Horticultural Science & Biotechnology 86:353-358. DOI: Doi 10.1080/14620316.2011.11512773.

Nanasato Y., Akashi K., Yokota A. (2005) Co-expression of cytochrome b_{561} and ascorbate oxidase in leaves of wild watermelon under drought and high light conditions. Plant Cell Physiol 46:1515-24. DOI: 10.1093/pcp/pci164.

Nawaz M.A., Chen C., Shireen F., Zheng Z., Sohail H., Afzal M., Ali M.A., Bie Z., Huang Y. (2018) Genome-wide expression profiling of leaves and roots of watermelon in response to low nitrogen. BMC Genomics 19:456. DOI: 10.1186/s12864-018-4856-x.

Ngwepe R.M., Mashilo J., Shimelis H. (2019) Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides*): a review. Genetic Resources and Crop Evolution 66:735-758. DOI: 10.1007/s10722-018-0724-4.

Omirou M., Ioannides I.M., Ehaliotis C. (2013) Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots, but leads to improved colonization and plant response under water stress only. Applied Soil Ecology 63:112-119. DOI: 10.1016/j.apsoil.2012.09.013.

Paliyath G., Bakovic M., Shetty K. (2011) Functional foods, nutraceuticals, and degenerative disease prevention John Wiley & Sons.

Pandey K.B., Rizvi S.I. (2009) Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2:270-278. DOI: DOI 10.4161/oxim.2.5.9498.

Paris H.S. (2016) Overview of the origins and history of the five major cucurbit crops: issues for ancient DNA analysis of archaeological specimens. Vegetation History and Archaeobotany 25:405-414. DOI: 10.1007/s00334-016-0555-1.

Park M., Han J., Ahn Y., Kim J., Lee H., Jang Y., Gaxiola R., Hirschi K.D., Park S. (2014) Ectopic expression of Arabidopsis H^+-pyrophosphatase AVP1 enhances drought resistance in bottle gourd (*Lagenaria siceraria* Standl.). Plant Cell Tissue and Organ Culture 118:383-389. DOI: 10.1007/s11240-014-0490-8.

Pasandide B., Khodaiyan F., Mousavi Z., Hosseini S.S. (2018) Pectin extraction from citron peel: optimization by Box-Behnken response surface design. Food Sci Biotechnol 27:997-1005. DOI: 10.1007/s10068-018-0365-6.

Ram C., Verma M., Panwar B.S., Arya L. (2015) Molecular cloning and in silico analysis of DREB-like gene in watermelon. Indian Journal of Horticulture 72:338-342. DOI: 10.5958/0974-0112.2015.00066.3.

Ramirez A.H.M., Jhala A.J., Singh M. (2014) Factors Affecting Germination of Citronmelon (*Citrullus lanatus* var. *citroides*). Weed Science 62:45-50. DOI: 10.1614/Ws-D-13-00041.1.

Rouphael Y., Kyriacou M.C., Colla G. (2017) Vegetable Grafting: A Toolbox for Securing Yield Stability under Multiple Stress Conditions. Front Plant Sci 8:2255. DOI: 10.3389/fpls.2017.02255.

Saha I., Sarkar J.P., Maulik U. (2019) Integrated Rough Fuzzy Clustering for Categorical data Analysis. Fuzzy Sets and Systems 361:1-32. DOI: 10.1016/j.fss.2018.02.007.

Sharma A., Shahzad B., Kumar V., Kohli S.K., Sidhu G.P.S., Bali A.S., Handa N., Kapoor D., Bhardwaj R., Zheng B. (2019) Phytohormones Regulate Accumulation of Osmolytes Under Abiotic Stress. Biomolecules 9. DOI: 10.3390/biom9070285.

Shepard D. (2014) Africa to push development agenda at upcoming climate summit. Africa Renewal 28:26-29.

Sherrard M.E., Maherali H. (2006) The adaptive significance of drought escape in *Avena barbata*, an annual grass. Evolution 60:2478-89. DOI: 10.1111/j.0014-3820.2006.tb01883.x.

Si Y., Dane F., Rashotte A., Kang K., Singh N.K. (2010) Cloning and expression analysis of the Ccrboh gene encoding respiratory burst oxidase in *Citrullus colocynthis* and grafting onto *Citrullus lanatus* (watermelon). J Exp Bot 61:1635-42. DOI: 10.1093/jxb/erq031.

Si Y., Zhang C., Meng S., Dane F. (2009) Gene expression changes in response to drought stress in *Citrullus colocynthis*. Plant Cell Rep 28:997-1009. DOI: 10.1007/s00299-009-0703-5.

Sultana B., Ashraf R. (2019) Watermelon (Citrullus lanatus) Oil, Fruit Oils: Chemistry and Functionality, Springer. pp. 741-756.

Swislocki M. (2008) Culinary nostalgia: Regional food culture and the urban experience in Shanghai Stanford University Press.

Takahara K., Akashi K., Yokota A. (2005) Biochemical and molecular characterization of glutamate N-acetyltransferase involved in citrulline accumulation in wild watermelon during drought/strong-light stresses. Plant and Cell Physiology 46:S74-S74.

Takahara K., Yoshino A., Akashi K., Yokota A. (2006) Regulation of metabolic pathways for the massive accumulation of citrulline during drought/strong light stress in wild watermelon. Plant and Cell Physiology 47:S102-S102.

Tardieu F. (2013) Plant response to environmental conditions: assessing potential production, water demand, and negative effects of water deficit. Front Physiol 4:17. DOI: 10.3389/fphys.2013.00017.

Ujoh F., Igbawua T., Paul M.O. (2019) Suitability mapping for rice cultivation in Benue State, Nigeria using satellite data. Geo-Spatial Information Science 22:332-344. DOI: 10.1080/10095020.2019.1637075.

Unel N.M., Cetin F., Karaca Y., Altunoglu Y.C., Baloglu M.C. (2019) Comparative identification, characterization, and expression analysis of bZIP gene family members in watermelon and melon genomes. Plant Growth Regulation 87:227-243. DOI: 10.1007/s10725-018-0465-6.

Wei C., Zhang R., Yang X., Zhu C., Li H., Zhang Y., Ma J., Yang J., Zhang X. (2019) Comparative Analysis of Calcium-Dependent Protein Kinase in Cucurbitaceae and Expression Studies in Watermelon. Int J Mol Sci 20. DOI: 10.3390/ijms20102527.

Xu Z., Sun M., Jiang X., Sun H., Dang X., Cong H., Qiao F. (2018) Glycinebetaine Biosynthesis in Response to Osmotic Stress Depends on Jasmonate Signaling in Watermelon Suspension Cells. Front Plant Sci 9:1469. DOI: 10.3389/fpls.2018.01469.

Yang J., Zhu J.H., Yang Y.X. (2017) Genome-Wide Identification and Expression Analysis of NF-Y Transcription Factor Families in Watermelon (Citrullus lanatus). Journal of Plant Growth Regulation 36:590-607. DOI: 10.1007/s00344-017-9670-1.

Yang X., Li H., Yang Y., Wang Y., Mo Y., Zhang R., Zhang Y., Ma J., Wei C., Zhang X. (2018) Identification and expression analyses of WRKY genes reveal their involvement in growth and abiotic stress response in watermelon (*Citrullus lanatus*). PLoS One 13:e0191308. DOI: 10.1371/journal.pone.0191308.

Yokota A., Kawasaki S., Iwano M., Nakamura C., Miyake C., Akashi K. (2002) Citrulline and DRIP-1 protein (ArgE homologue) in drought tolerance of wild watermelon. Ann Bot 89 Spec No:825-32. DOI: 10.1093/aob/mcf074.

Yoshida K., Akashi K., Yokota A. (2005) Proteomic analysis of drought/strong light stress responses in wild watermelon leaves. Plant and Cell Physiology 46:S72-S72.

Yoshimura K., Masuda A., Akashi K., Yokota A. (2005) Identification of drought-responsible proteins in the root of wild watermelon by proteomic analysis. Plant and Cell Physiology 46:S74-S74.

Yoshimura K., Masuda A., Akashi K., Yokota A. (2006) Regulation of the root development mechanism involved in Ran GTPase of wild watermelon under drought stress. Plant and Cell Physiology 47:S101-S101.

Yoshimura K., Masuda A., Kuwano M., Yokota A., Akashi K. (2008) Programmed proteome response for drought avoidance/tolerance in the root of a C(3) xerophyte (wild watermelon) under water deficits. Plant Cell Physiol 49:226-41. DOI: 10.1093/pcp/pcm180.

Yu C., Dong W., Zhan Y., Huang Z.A., Li Z., Kim I.S., Zhang C. (2017) Genome-wide identification and expression analysis of ClLAX, ClPIN and ClABCB genes families in *Citrullus lanatus* under various abiotic stresses and grafting. BMC Genet 18:33. DOI: 10.1186/s12863-017-0500-z.

Zabel F., Putzenlechner B., Mauser W. (2014) Global agricultural land resources--a high resolution suitability evaluation and its perspectives until 2100 under climate change conditions. PLoS One 9:e107522. DOI: 10.1371/journal.pone.0107522.

Zadawa A.N., Omran A. (2020) Rural Development in Africa: Challenges and Opportunities, Sustaining our Environment for Better Future, Springer. pp. 33-42.

Zhang H.S., Yang Y.G., Su Y.Q., Li X.F. (2017) Antioxidant enzymes activities in leaves and yield analysis of different ecological types watermelon under drought stress. Agricultural Research in the Arid Areas 35:138-143. DOI: 10.7606/j.issn.1000-7601.2017.03.22.

Zhengkang H., Wang G., Yao W., Zhu W.-y. (2006) Isoflavonic phytoestrogens-new prebiotics for farm animals: a review on research in China. Current issues in intestinal microbiology 7:53-60.

Zhou Y., Li J.W., Wang J.H., Yang W.T., Yang Y.X. (2018) Identification and Characterization of the Glutathione Peroxidase (GPX) Gene Family in Watermelon and Its Expression under Various Abiotic Stresses. Agronomy-Basel 8. DOI: 10.3390/agronomy8100206.

Chapter 3: Nutrient composition and physical properties of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) seeds are related to seed coat visual appearance

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Abstract

Roasted citron watermelon seeds are eaten as snacks based on maturity indices. Association between coat colour, nutrient composition and physical properties is unknown. Therefore, seed coat thickness (SCT), hardness and popping yield (PY) may influence roasting quality and chewability. Understanding seed nutrient composition, physical properties, and coat colour associations allows selection for desirable traits. Forty accessions were analysed for mineral elements using flame atomic emission spectrometry (FAES) for K and flame atomic absorption spectrometry (FAAS) for Mg, Cu, Mn, and Zn. Physical properties such as (seed thickness (ST), weight required to crack seed (WRCS), SCT, and PY were analysed. Data was subjected to correlation and Principal Component Analysis. Significant differences were observed for mineral elements and physical properties ($P < 0.05$). Dark and red seeds had high ST and SCT but low WRCS, Cu and Zn compared to brown seeds. Significantly lower PY and high Ca content were recorded for dark seeds. Dark seeds had high hemicellulose (19.58%), cellulose (39.17%) and lignin (30.08%). Popping yield was positively correlated with lightness ($r = 0.72$; $P < 0.001$) and negatively correlated with Ca ($r = -0.77$; $P < 0.001$). Seeds with lighter colours had higher popping yield and less Ca, implying that coat colour influences palatability, nutrient composition and physical properties. Study findings will guide breeding strategies to enhance the nutritive value of seeds.

Keywords: Hidden hunger, Micronutrients, Nutritional quality, Principal component analysis (PCA), Snack, Sustainable Development Goal 2

3.1 Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) is an underutilized crop grown by small-holder farmers in sub-Saharan Africa (SSA). The fresh and tender leaves are eaten as green leafy vegetables. The fruit's flesh is boiled and mixed with maize meal to cook porridge “Kgodu” in South Africa and Botswana (Bultosa et al., 2020). Mature seeds extracted from the fruit are sun-dried, roasted and consumed as a snack (Mandizvo et al., 2021). The seeds are a rich source of protein, with protein fractions including globulins (63.7%), albumins (18.6%) and glutelins (14.0%) (Singh and Matta, 2010). The seeds' oil content ranges from 22 to 50% (Jarret and Levy, 2012) and contains > 80% unsaturated fatty acids and < 20% saturated fatty acids, with linoleic acid being the abundant (68.3%) fatty acid (Mabaleha et al., 2006). Jarret and Levy (2012) reported high linoleic acid content (63.37%), followed by oleic acid (16.42%), palmitic acid (10.6%) and stearic acid (8.05%).

The nutritive value (in terms of mineral elements) of citron watermelon places this crop among novel species with potential to reduce micronutrient deficiency (hidden hunger) common in SSA (Chacha and Laswai, 2020). Consuming citron watermelon seeds is influenced by their palatability, primarily determined by chewability and flavour after roasting. Also, the choice of which type of seed to eat is based on seed coat colour. White or cream seeded seeds have soft seed coats which are easy to chew when roasted. Red-white and red seeds have medium-hard seed coats which are crunchy and easy to chew after roasting. On the contrary, seeds with heavy pigmentation (black and maroon) are not easy to chew, often not preferred for roasting, and are less palatable (Nkoana et al., 2021). The variability exhibited in the ease with which citron watermelon is chewable is probably related to seed physical characteristics such as seed coat thickness and structural composition (lignin and cellulose content).

The species’ germplasm exhibits wide variation with respect to seed morphotypes, including seed characteristics. Seed characteristics such as seed coat colour, mechanical parameters, rheological properties, and nutritional composition provide an excellent measure of palatability. Seeds display compositional variations, inhomogeneities, and anisotropic structures (Nguyen et al., 2018). Joshi et al. (1993), divided the seed physical properties into (i) thermal properties (thermal transfer and diffusivity), (ii) optical properties (colour, gloss and translucency), (iii) structural and geometrical properties (mass, density, particle size, shape and cellularity) and (iv) mechanical properties (ductility, compressibility, malleability and rheology). For citron watermelon, seed properties may serve as an important selection criterion for cultivar selection targeting improvement of nutritional and biochemical traits.

Recent studies have shown that, watermelon seeds can be used as a raw material for preparation of Robo “Nigerian snack” (Adeyeye et al., 2019). Zia et al. (2021) reported that watermelon seeds are an excellent source of protein (15–50%) such as albumin, globulin, prolamin, and glutelin. Information linking seed characteristics such as coat colour, thickness/hardness, and biochemical composition (lignin, cellulose and hemicellulose content) with nutritional profiles could be useful for breeding, developing nutritionally enhanced genotypes, consumption and processing value-added food products. However, there is limited information associating seed physical characteristics and biochemical composition with nutritional quality and palatability (taste, chewability), roasting and popping citron watermelon seeds. Therefore, seed-derived products' processing and value-addition in citron watermelon require understanding the seed's physical, mechanical, and chemical properties.

The objectives of this study were, (i) to determine whether physical characteristics, biochemical and nutrient compositions in mature seeds of genetically distinctive genotypes of citron watermelon with varied seed coat colours; and (ii) to test whether seed physical characteristics (coat colour) and biochemical compositions (cellulose and lignin content) are associated with citron watermelon nutritive quality, palatability (roasting, taste and ease of chewing) and popping yield.

3.2 Materials and methods

3.2.1 Plant material

Forty genetically distinct citron watermelon landrace accessions were sourced from the Limpopo Department of Agriculture and Rural Development, Towoomba Research Station, Bela-Bela, Limpopo Province, South Africa. The selected accessions grown widely in the Limpopo Province were planted under field conditions at Towoomba Research Station during the 2017-18 growing seasons. At maturity, seeds were extracted, sun-dried and stored for further analysis. The codes, seed shape and seed coat colours of the citron watermelon accessions are shown in Figure 3.1.

Figure 3.1:Codes and seed coat colours of citron watermelon landrace accessions used in the study

3.2.2 Physical properties

Seed traits including seed length (SL), seed width (SW), seed thickness (ST), hundred seed weight (HSW) and moisture content (MC) were measured in five replications at the Seed Technology Laboratory, University of KwaZulu-Natal, South Africa. Seed length and SW were measured using a digital calliper (150 mm (6"), China) (Supplementary Figure 1a). A digital balance (Sartorius AG, CPA1245, Germany) with a readability of 0.001 g was used to measure HSW (Supplementary Figure 1b). An Intelligent Grain Moisture meter, KM-21G (Pretoria, South Africa), was used to estimate the seed MC (Supplementary Figure 1c).

3.2.3 Seed coat colour

Seed coat colour was measured using a stereomicroscope integrated with computer software (Leica Application Suite 4.0). Seeds from each accession were placed on a white Whatman filter paper background on the stereomicroscope stage. The stage was set at a 25 mm working distance. Measurements of red, green and blue (RGB) were recorded for each seed in triplicate for each accession using a different set of seeds. Images were captured using Leica DFC 450C camera integrated with the stereomicroscope.

3.2.4 Hue, saturation and lightness (HSL)

Converting tri-stimulus values (RGB) to HSL was performed according to (Mandizvo and Odindo, 2019a) by standardizing RGB values (Equation 1, 2, 3, 4, 5 and 6).

$$nr = \frac{R}{R + G + B} \qquad (1)$$

$$ng = \frac{G}{R + G + B} \qquad (2)$$

$$nb = \frac{B}{R + G + B} \qquad (3)$$

Where nr, ng and nb are normalized values between 0 and 1, with $nr + ng + nb = 1$. The transformation to HSL was achieved by using the following equations:

$$\boldsymbol{H} = [2\pi] - \cos^{-1}\left(\frac{0.5 \times ((nr - ng) + (nr - nb))}{\sqrt{((nr - ng)^2) + (nr - nb)(ng - nb))}}\right) \qquad (4)$$

$$\boldsymbol{S} = 1 - 3 \times minimum\ (nr, ng, nb) \qquad (5)$$

$$\boldsymbol{L} = \frac{R + G + B}{3 \times 255} \qquad (6)$$

3.2.5 *Seed coat thickness*

Seed coat thickness was measured using a Zeiss-EVO scanning electron microscope (SEM) (Zeiss, Oberkochen, Germany). Seeds were cryofractured in liquid N_2 and split into halves. Seeds were mounted on stubs and secured with carbon insulating tape. The seeds were gold-coated using a gold sputter coat (Quorum Q150R ES) and viewed under the Zeiss-EVO SEM in high vacuum mode. Images were captured on the SEM at 5 kV and seed coat thickness was measured using analysis software (Soft Imaging System, Münster, Germany).

3.2.6 *Mechanical properties*

Seed hardness was measured in triplicates using an Instron Wolpert Hardness Tester (Serie R-Testor ® 600) (Supplementary Figure 2). Seeds were loaded flat under the indenter which moved vertically. There were two load indicators: the black one turned due to pressure and went back to "zero" when the seed broke. The red one remained still after breaking the seed, indicating the breaking load or seed hardness. Seed hardness was expressed as kg force applied. The crushing strength was calculated (Equation 7) using the indenter's projected area, with radius of circular indenter set at 0.200 cm (Haque et al., 2010).

$$\text{Crushing Strength (kg cm}^{-2}\text{)} = \frac{\text{Weight required to crack seed}}{\text{The projected area of seed under load}} \qquad (7)$$

3.2.7 Seed mineral analysis

3.2.7.1 Sample preparation decimating

Dried seeds were ground into powder using a stainless-steel Wiley mill grinder. Ground samples (0.5 g) were put in quartz crucibles and charred in a Wild Barfield muffle furnace at 650 ºC for 120 minutes. Acid digestion was done according to Huang et al. (2007) with minor modifications. The ash was dissolved in 10 ml dilute *aqua regia* (HNO_3 and HCl mixed in ratio 1:3) in a 25 ml volumetric flask. Contents of the flask were brought to the volume by adding distilled H_2O and heated in a water bath for 1 hour at 85 ºC. Samples were chilled to 25 ºC before analysis by AAS (Mandizvo and Odindo, 2019b).

3.2.7.2 Atomic absorption spectrometry

Mineral element (Ca, Cu, Fe, K, Mg, Mn, and Zn) analysis was done using Fast Sequential Absorption Spectrometer (FSAS) (Varian AA280FS) hyphenated to computer software (Spectr AA version 5.1 PRO). The AAS was calibrated using an ICP multi-element standard solution IV prepared within the flame absorbance and emission spectrophotometer's operating range (0–100 ppm). The photometer's flame was calibrated by adjusting air (12 L/min) and gas (8 L/min) flow. After allowing the flame hollow cathode lamp to settle for 5 minutes, the lamp was calibrated to emit stable radiation. Readings of the galvanometer were adjusted to zero by spraying blank 5% HNO_3 into the flame. Sensitivity was adjusted by spraying standard solutions into the flame to get a full-scale deflection of the galvanometer. Sample solutions were aspirated into the flame three times and the readings of the galvanometer were recorded. Elemental concentrations in each sample were calculated from the graph of concentration against absorbance. The final concentration was calculated using sample mass and volume (Equation 8).

$$\text{Final concentration} = \text{Average} \times \frac{\text{Volume (25 ml)}}{\text{mass (0.5 g)}} \qquad (8)$$

3.2.8 Carbon, nitrogen and sulfur determination

Seeds were analyzed in triplicates for carbon (C), nitrogen (N) and sulfur (S) using the LECO-Traumas CNS Autoanalyzer (LECO CNS-2000, Leco-cooperation, St Joseph MI, USA, 2012). The instrument was calibrated by analyzing; (i) three empty crucibles (ii) five crucibles with 0.2 g calibration sample (Ethylenediaminetetraacetic acid (EDTA) [Carbon% (41.02±0.26), Hydrogen% (5.51±0.03) and Nitrogen (9.56±0.06)] mixed with 1 g combustion catalyst (Com-Cat) accelerator (502-321)). Samples (0.2 g) of ground seeds were weighed into 528-203 crucibles and mixed with 1 g Com-Cat Accelerator. The crucibles were transferred to the autoloader before starting the analysis sequence. For sulfur analysis, the soil calibration sample for CNS [Carbon% (11.89±0.26), Nitrogen (0.94±0.03) and Sulphur% (0.139±0.004)] was used to condition the instrument before analysis.

3.2.9 Seed hulls hemicellulose, cellulose and lignin content

The contents of hemicellulose, cellulose and lignin in citron watermelon seed hulls were determined. Seed hulls were separated from the embryo (Supplementary Figure 3) by soaking seeds in distilled water for 120 hours (Wu and Shen, 2020). Seed hull samples were homogenized and ground to particles of <0.5 mm. Moisture was determined by heating at 105 °C to constant weight. Treatments with 0.255 N-H_2SO_4 and 0.13 N-NaOH were performed to determine crude fibre (CF) following (Hollmann et al., 2013). Neutral detergent fibre (NDF) values were obtained by refluxing the samples with sodium lauryl sulfate (pH 7.0) solution for 1 hour. Lignocellulosic biomass was determined as loss in weight of the NDF, following treatment with 72% H_2SO_4 and calcination at 550 °C to obtain hemicellulose, cellulose and lignin.

3.2.10 Determination of popping yield

Twenty seeds from each accession were popped separately in a microwave. Microwave popping was done using Hisense microwave oven (1 450 W, 50 Hz, 2450 MHz, H30MOMMI, China) at 900 W power setting to pop the seeds for 180 s. Partially hydrogenated *Helianthus annuus* oil (5 ml) was added to citron watermelon seeds. The mixture was distributed uniformly at the bottom of microwave popping ceramic bowl. The bowl was covered with a porcelain lid. A seed was considered popped when it shows a fissure (crack) after180 s. The number of popped and un-popped seeds was recorded for each sample.

3.2.11 Data analysis

Data collected were subjected to analysis of variance (ANOVA) using GenStat statistical analysis software 18th edition (VSN International, Hempstead, UK). Means were separated using the Fisher's protected least significant difference (LSD) when treatments showed significant differences at 5% level of probability. Correlation analysis was performed to determine associations between the seed's physical and nutritional properties using the *corrplot* package in R version 4.0 (R Core Team, 2020). Principal component analysis (PCA) and the Kaiser-Meyer-Olkin measure of sampling adequacy were performed based on the correlation matrix using XLSTAT (XLSTAT 2020.5.1.1075). The loading factors derived from the PCA were used to identify variables that have a strong relationship with a particular PC. Principal component biplots were created to examine correlations between citron watermelon accessions and nutritional and physical attributes to guide the selection of suitable genotypes.

3.3 Results

3.3.1 Physical properties of citron watermelon seed

Significant ($P < 0.05$) differences were recorded for seed physical properties (seed length, seed width, seed ratio, seed thickness and hundred seed weight) among the studied accessions (Table 3.1). For seed length, accessions WWM-24, WWM-39, WWM-45(A) and WWM-05 had longer seeds (> 12.000 mm). Conversely, accessions such as WWM-03, WWM-17(2), WWM-68, WWM-41(B) and WWM-02 had shorter seed length (< 11 mm). For seed width, accessions WWM-24, WWM-45(A) and WWM-28 had high seed width values (> 7.000 mm), whereas lower seed width values (< 6.000 mm) were recorded for accessions WWM-17(2) and WWM-03.

Seed thickness ranged from 2.12 to 2.81 mm, with genotypes WWM-79, WWM-05, WWM-81 and WWM-66 recording higher values of ≥ 2.750 mm. Accessions WWM-45(B), WWM-03, WWM-35(2), WWM-64 and WWM-41(A) had thinner seeds < 2.330 mm. Hundred seed weight (HSW) varied from 8.010 to 18.080 g, with accessions WWM-24, WWM-28, WWM-16 and WWM-45(A) recording higher values ≥ 15.340 g. Accessions WWM-45(B), WWM-64 and WWM-17(2) had lower HSW < 9.200 g. Percentage seed moisture content (MC) did not differ significantly ($P = 0.144$).

Table 3.1: Mean values for physical properties of 40 citron watermelon genotypes

Genotype	**SL (mm)**	**SW (mm)**	**S.R (L/W)**	**ST (mm)**	**HSW (g)**	**MC (%)**
WWM-02	10.570^{abc}	6.420^{cdef}	1.650^{abc}	2.570^{jklmnop}	11.010^{ij}	11.340^{cd}
WWM-03	**10.000^{a}**	5.870^{b}	1.710^{abcdef}	2.250^{b}	8.550^{abc}	11.370^{cd}
WWM-05	12.730^{opq}	7.390^{nop}	1.730^{abcdef}	2.360^{bcdef}	11.330^{jk}	**10.860^{a}**
WWM-07	12.230^{klmnopq}	6.770^{fghij}	1.810^{cdefg}	2.710^{qrst}	14.610^{opq}	11.050^{abcd}
WWM-08	12.230^{klmnopq}	7.000^{hijklmn}	1.750^{abcdef}	2.750st	15.110^{pqr}	11.260^{bcd}
WWM-09	12.590^{nopq}	6.960^{hijkl}	1.810^{defg}	2.580^{lmnopqr}	14.000^{mno}	11.330^{bcd}
WWM-14	11.740^{ghijklm}	7.180^{klmno}	1.640^{ab}	2.590^{lmnopqr}	15.000^{pqr}	11.010^{abc}
WWM-15	12.220^{klmnopq}	7.360^{mnop}	1.660^{abcd}	2.700^{pqrst}	14.470^{nop}	11.170^{abcd}
WWM-16	12.190^{jklmnop}	7.110^{ijklmno}	1.720^{abcdef}	2.640^{nopqrs}	15.440^{r}	11.170^{abcd}
WWM-17(2)	10.060^{a}	**5.460^{a}**	1.850^{fg}	2.430^{defghij}	8.520^{ab}	11.120^{abcd}
WWM-21	11.430^{defghi}	6.860^{ghijk}	1.670^{abcd}	2.620^{nopqrs}	12.340^{l}	11.370^{cd}
WWM-23	11.550^{efghijk}	6.540^{defg}	1.770^{bcdefg}	2.670^{opqrs}	11.070^{ij}	11.240^{abcd}
WWM-24	**15.880^{r}**	**8.360^{q}**	**1.920^{g}**	2.570^{klmnop}	**18.080^{s}**	11.150^{abcd}
WWM-28	12.430^{mnopq}	7.490^{op}	1.660^{abcd}	2.600^{mnopqr}	15.440^{r}	11.140^{abcd}
WWM-33	10.910^{bcdef}	6.320^{cde}	1.730^{abcdef}	2.440^{efghijk}	9.280^{de}	11.020^{abc}
WWM-34	10.750^{bcd}	6.180^{bcd}	1.740^{abcdef}	2.590^{lmnopqr}	10.220^{fgh}	11.010^{abc}
WWM-35(1)	11.300^{defgh}	6.530^{defg}	1.730^{abcdef}	2.510^{ghijklmn}	10.220^{fgh}	**11.420^{d}**
WWM-35(2)	10.950^{bcdef}	6.470^{defg}	1.700^{abcdef}	2.260^{b}	10.440^{ghi}	11.080^{abcd}
WWM-37(2)	12.010^{hijklmno}	6.530^{defg}	1.840^{efg}	2.530^{hijklmno}	12.400^{l}	11.330^{bcd}
WWM-38	10.850^{bcdef}	6.470^{defg}	1.680^{abcd}	2.470^{fghijklm}	10.560^{hi}	11.130^{abcd}
WWM-39	12.920^{q}	7.400^{op}	1.750^{abcdef}	2.570^{klmnopq}	15.171^{pqr}	11.120^{abcd}
WWM-40	10.830^{bcde}	6.070^{bc}	1.790^{bcdefg}	2.350^{bcdef}	9.870^{defgh}	11.270^{bcd}
WWM-41(A)	11.690^{ghijkl}	6.730^{fghi}	1.740^{abcdef}	2.310^{bcd}	11.170^{ij}	11.020^{abc}
WWM-41(B)	10.460^{ab}	6.290^{cde}	1.660^{abcd}	2.380^{bcdefg}	9.690^{defg}	11.360cd
WWM-44	11.390^{defghi}	6.510^{defg}	1.750^{abcdef}	2.460^{efghijkl}	10.430^{ghi}	10.970^{abc}
WWM-45(A)	12.830^{pq}	7.570^{p}	1.700^{abcdef}	2.700^{pqrst}	15.340^{qr}	11.220^{abcd}
WWM-45(B)	10.750^{bcd}	6.730^{fghi}	**1.600^{a}**	**2.120^{a}**	**8.010^{a}**	11.090^{abcd}
WWM-46	12.310^{lmnopq}	7.290^{lmnop}	1.690^{abcdef}	2.710^{rst}	13.460^{m}	11.080^{abcd}
WWM-47	12.170^{jklmnop}	6.980^{hijklm}	1.750^{abcdef}	2.700^{pqrst}	13.810^{mn}	11.180^{abcd}
WWM-50	11.550^{efghijk}	6.760^{fghij}	1.710^{abcdef}	2.380^{bcdefg}	11.670^{jkl}	11.100^{abcd}
WWM-57	11.470^{defghij}	6.630^{efgh}	1.730^{abcdef}	2.570^{klmnop}	11.120^{ij}	11.240^{abcd}
WWM-64	11.570^{fghijk}	6.640^{efgh}	1.740^{abcdef}	2.300^{bc}	8.440^{a}	11.130^{abcd}
WWM-64(2)	11.860^{ghijklmn}	7.260^{lmnop}	1.640^{ab}	2.330^{bcde}	9.530^{def}	11.140^{abcd}
WWM-65	12.060^{ijklmno}	7.220^{klmnop}	1.670^{abcd}	2.510^{ghijklmn}	12.380^{l}	11.020^{abc}
WWM-66	12.500^{nopq}	7.130^{jklmno}	1.760^{abcdef}	2.750st	13.680^{m}	11.250^{abcd}
WWM-67	11.240^{cdefg}	6.280^{cde}	1.790^{bcdefg}	2.430^{cdefghi}	9.200^{bcd}	11.060^{abcd}
WWM-68	10.070^{a}	6.150^{bcd}	1.640^{ab}	2.410^{cdefgh}	9.240^{cde}	11.120^{abcd}
WWM-76	11.930^{ghijklmn}	7.330^{lmnop}	1.630^{ab}	2.550^{ijklmno}	13.400^{m}	11.120^{abcd}
WWM-79	11.690^{ghijkl}	6.510^{defg}	1.800^{bcdefg}	**2.810^{t}**	11.920^{kl}	10.940^{ab}
WWM-81	10.860^{bcdef}	6.450^{def}	1.680^{abcde}	2.750st	9.980^{efgh}	11.090^{abcd}
L.S.D(0.05)	0.607	0.326	0.128	0.114	0.658	0.311
CV%	0.800	0.800	1.300	0.400	4.40o	2.201
Skewness	1.307	0.500	0.690	-0.220	-0.440	-1.280
Kurtosis	5.890	1.370	2.370	-0.360	-0.540	-0.310
P-value	<.001	<.001	<.001	<.001	<.001	0.1440

*Means in the same column followed by the same letter are not significantly different, while figures with different footnotes are significantly different according to Fisher's test ($P<0.05$)

SL; Seed Length, **SW**; Seed Width, **S.R**; Slender Ratio. **ST**; Seed Thickness, **HSW**; Hundred Seed Weight, **MC;** Seed Moisture Content

3.3.2 Seed coat colour

Saturation and lightness were significantly different among accessions (P < 0.05). The ranges for saturation and lightness were 13.33-100% and 10-100%, respectively. Accessions WWM-45(A) and WWM-65 recorded the highest saturation of 100%. Lowest saturation value (13.33%) was recorded for accession WWM-79. Accessions WWM-79, WWM-03 and WWM-14 recorded higher lightness values ≥ 98.670%, whereas WWM-21, WWM-07 and WWM-24 had the lower lightness ≤ 35.670% (Table 3.2). Twenty-two technical seed coat colours were identified using the Reichs-Ausschuß für Lieferbedingungen und Gütesicherung (RAL) colour system. The colour bright red-orange had highest frequency (5) among the studied accessions (Figure 3.2).

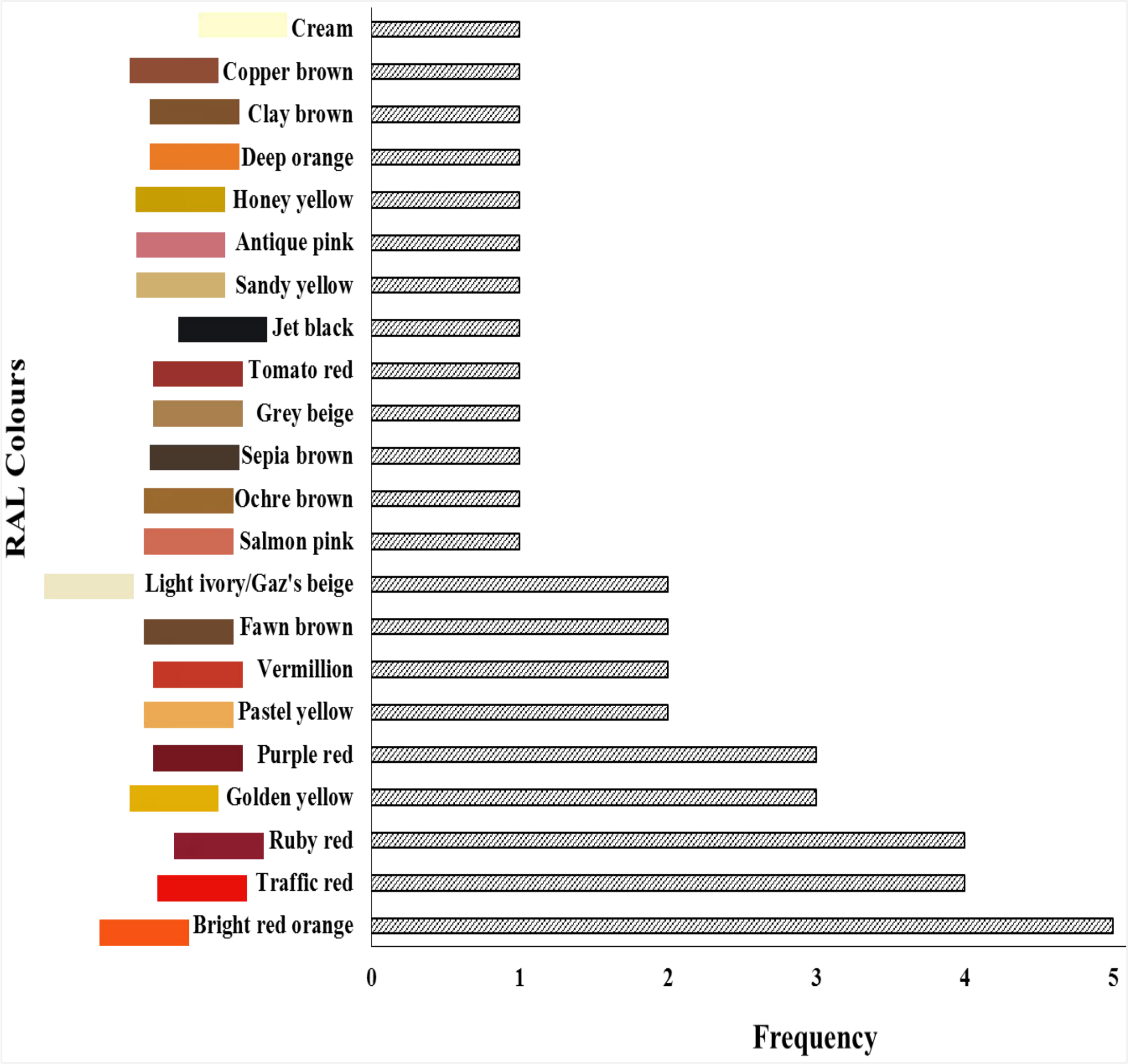


Figure 3.2: Frequency distribution of seed coat colour among 40 citron watermelon accessions based on the Reichs-Ausschuß für Lieferbedingungen und Gütesicherung colour system

Table 3.2: Hue, saturation, lightness and RAL colour codes for seed of citron watermelon accessions

Genotype	**Hue (°)**	**Saturation (%)**	**Lightness (%)**	**RAL**	**Colour Name**
WWM-02	13.000^{a}	76.67^{fg}	88.670^{jkl}	3022	Salmon pink
WWM-03	26.000^{a}	55.67^{bc}	100.000^{m}	1034	Pastel yellow
WWM-05	33.670^{a}	81.00^{fgh}	58.000^{f}	8001	Ochre brown
WWM-07	24.000^{a}	79.33^{fg}	26.330^{b}	8014	Sepia brown
WWM-08	8.670^{a}	95.67^{jkl}	85.000^{ij}	2002	Vermillion
WWM-09	34.330^{a}	56.67^{bc}	55.000^{f}	1011	Grey beige
WWM-14	15.670^{a}	81.67^{fghi}	99.330^{m}	2008	Bright red orange
WWM-15	7.000^{a}	96.67^{kl}	60.330^{f}	3013	Tomato red
WWM-16	5.000^{a}	99.00^{l}	75.670^{gh}	3020	Traffic red
WWM-17(2)	32.330^{a}	72.000^{def}	86.000^{jk}	1004	Golden yellow
WWM-21	24.670^{a}	93.670^{ijkl}	10.000^{a}	9005	Jet black
WWM-23	16.000^{a}	85.670^{ghijk}	97.670^{m}	2008	Bright red orange
WWM-24	39.330^{a}	81.000^{fgh}	32.670^{bc}	8007	Fawn brown
WWM-28	3.330^{a}	98.330^{l}	76.670^{ghi}	3020	Traffic red
WWM-33	30.330^{a}	46.330^{b}	99.000^{m}	1015	Light ivory/Gaz's beige
WWM-34	31.670^{a}	53.670^{bc}	84.000^{hij}	1002	Sandy yellow
WWM-35(1)	2.670^{a}	99.330^{l}	55.670^{f}	3003	Ruby red
WWM-35(2)	32.670^{a}	75.670^{efg}	85.330^{j}	1004	Golden yellow
WWM-37(2)	0.670^{a}	97.330^{kl}	38.670^{cde}	3004	Purple red
WWM-38	15.670^{a}	83.000^{fghi}	94.670^{lm}	2008	Bright red orange
WWM-39	120.33^{a}	99.670^{l}	58.330^{f}	3003	Ruby red
WWM-40	10.330^{a}	96.330^{kl}	85.000^{ij}	2002	Vermillion
WWM-41(A)	120.00^{a}	99.330^{l}	41.000^{cde}	3004	Purple red
WWM-41(B)	13.670^{a}	79.670^{fg}	97.670^{m}	2008	Bright red orange
WWM-44	9.330^{a}	63.000^{cd}	97.670^{m}	3014	Antique pink
WWM-45(A)	0.330^{a}	100.000^{l}	60.000^{f}	3003	Ruby red
WWM-45(B)	32.00^{a}	63.670^{cde}	98.33^{m}	1034	Pastel yellow
WWM-46	0.670^{a}	99.000^{l}	60.670^{f}	3003	Ruby red
WWM-47	2.330^{a}	98.330^{l}	73.670^{g}	3020	Traffic red
WWM-50	2.330^{a}	98.330^{l}	72.330^{g}	3020	Traffic red
WWM-57	32.670^{a}	73.670^{defg}	82.330^{hij}	1004	Golden yellow
WWM-64	32.670^{a}	92.670^{hijkl}	72.000^{g}	1005	Honey yellow
WWM-64(2)	31.330^{a}	84.000^{fghij}	83.670^{hij}	2011	Deep orange
WWM-65	0.000^{a}	100.000^{l}	42.670^{de}	3004	Purple red
WWM-66	21.000^{a}	72.330^{def}	45.000^{e}	8003	Clay brown
WWM-67	14.670^{a}	83.000^{fghi}	94.000^{klm}	2008	Bright red orange
WWM-68	43.330^{a}	76.000^{fg}	35.670^{cd}	8007	Fawn brown
WWM-76	23.330^{a}	82.330^{fghi}	55.670^{f}	8004	Copper brown
WWM-79	57.330^{a}	13.330^{a}	100.000^{m}	9001	Cream
WWM-81	33.000^{a}	48.330^{b}	98.670^{m}	1015	Light ivory/ Gaz's beige
P-value	0.552	<.001	<.001	-	-
LSD$_{(0.05)}$	75.510	5.961	4.216	-	-

*Means in the same column followed by the same letter are not significantly different, while figures with different letters are significantly different according to Fisher's test (P<0.05). LSD = Least Significance Difference ***RAL**; Reichs-Ausschuß für Lieferbedingungen und Gütesicherung

3.3.3 Seed coat thickness, mechanical properties and popping yield

Significant differences in seed coat thickness were observed among accessions ($P<0.001$). Accessions WWM-79 and WWM-03 had lower seed coat thickness (< 131 µm) while accessions WWM-64, WWM-21 and WWM-07 had higher seed coat thickness (> 135.500 µm) (Table 3.3). Micrographs of seed coat thickness measurements under SEM are shown in Figure 3.3. Weight required to crack seed (WRCS) and crushing strength (CS) significantly differ among the studied accessions ($P<0.01$). Highest WRCS (0.623 kg) and CS (4.958 kgcm^{-2}) were recorded for WWM-64(2). Lowest WRCS (0.184 kg) and CS (1.467 kgcm^{-2}) were recorded for accession WWM-79. The popping yield of seeds differ significantly ($P<0.001$) among accessions. WWM-79, WWM-03 and WWM-40 had higher popping yield (≥ 85%). Lower popping yield (< 55%) was recorded for accessions WWM-21, WWM-68 and WWM-37(2).

Table 3.3: Seed coat thickness and mechanical properties of 40 citron watermelon accessions with varied seed coat colours

Genotype	**SCT (µm)**	**WRCS (kg)**	**CS (kg cm^{-2})**	**PY (%)**
WWM-02	131.600^{bcde}	0.255^{de}	2.032^{de}	81.670^{efgh}
WWM-03	130.400^{ab}	0.257^{def}	2.042^{def}	90.000^{gh}
WWM-05	133.900^{ghi}	0.276^{j}	2.194^{j}	68.330^{bcdefgh}
WWM-07	134.500^{ij}	0.315^{m}	2.509^{m}	55.000^{abcd}
WWM-08	132.100^{def}	0.262^{fgh}	2.085^{fgh}	75.000^{bcdefgh}
WWM-09	134.400^{ij}	0.286^{k}	2.273^{k}	66.670^{abcdefg}
WWM-14	130.500^{abc}	0.266^{hi}	2.119^{hi}	83.330^{efgh}
WWM-15	133.800^{ghi}	0.302^{l}	2.401^{l}	75.000^{bcdefgh}
WWM-16	133.600^{ghi}	0.282^{k}	2.247^{k}	68.330^{bcdefgh}
WWM-17(2)	131.600^{bcde}	0.196^{b}	1.560^{b}	80.000^{defgh}
WWM-21	134.600^{ij}	0.317^{m}	2.520^{m}	41.670^{a}
WWM-23	131.600^{bcde}	0.257^{def}	2.042^{def}	81.670^{efgh}
WWM-24	134.300^{ij}	0.306^{l}	2.435^{l}	55.000^{abcd}
WWM-28	133.500^{ghi}	0.284^{k}	2.257^{k}	75.000^{bcdefgh}
WWM-33	131.200^{abcd}	0.251^{d}	2.000^{d}	80.000^{defgh}
WWM-34	132.800^{efg}	0.186^{a}	1.483^{a}	70.000^{bcdefgh}
WWM-35(1)	134.300^{ij}	0.287^{k}	2.287^{k}	58.330^{abcde}
WWM-35(2)	131.600^{bcde}	0.193^{b}	1.536^{b}	78.330^{defgh}
WWM-37(2)	134.200^{hij}	0.317^{m}	2.525^{m}	51.670^{abc}
WWM-38	131.600^{bcde}	0.258^{efg}	2.050^{efg}	80.000^{defgh}
WWM-39	134.000^{ghij}	0.286^{k}	2.273^{k}	61.670^{abcdef}
WWM-40	131.800^{cdef}	0.257^{ef}	2.045^{ef}	85.000^{fgh}
WWM-41(A)	133.900^{ghi}	0.313^{m}	2.491^{m}	61.670^{abcdef}
WWM-41(B)	131.500^{bcd}	0.268^{i}	2.130^{i}	81.670^{efgh}
WWM-44	131.400^{abcd}	0.288^{k}	2.289^{k}	80.000^{defgh}
WWM-45(A)	133.800^{ghi}	0.286^{k}	2.273^{k}	66.670^{abcdefg}
WWM-45(B)	131.400^{abcd}	0.196^{b}	1.560^{b}	80.000^{defgh}
WWM-46	133.800^{ghi}	0.288^{k}	2.289^{k}	66.670^{abcdefg}
WWM-47	133.600^{ghi}	0.285^{k}	2.265^{k}	65.000^{abcdefg}
WWM-50	133.700^{ghi}	0.287^{k}	2.284^{k}	65.000^{abcdefg}
WWM-57	133.000^{fgh}	0.195^{b}	1.549^{b}	68.33^{bcdefgh}
WWM-64	135.200^{j}	0.218^{c}	1.737^{c}	76.6700^{cdefgh}
WWM-64(2)	132.800^{efg}	0.623^{n}	4.958^{n}	73.330^{bcdefgh}
WWM-65	134.100^{hij}	0.288^{k}	2.289^{k}	61.670^{abcdef}
WWM-66	134.200^{hij}	0.274^{j}	2.178^{j}	61.670^{abcdef}
WWM-67	131.500^{bcd}	0.263^{ghi}	2.093^{ghi}	76.670^{cdefgh}
WWM-68	134.200^{hij}	0.301^{l}	2.398^{l}	50.000^{ab}
WWM-76	134.200^{hij}	0.266^{hi}	2.114^{hi}	63.330^{abcdef}
WWM-79	130.200^{a}	0.184^{a}	1.467^{a}	93.330^{h}
WWM-81	131.300^{abcd}	0.186^{a}	1.483^{a}	78.330^{defgh}
P-value	<.001	<.001	<.001	<.001
LSD $_{(0.05)}$	0.632	2.767×10^{-3}	2.202×10^{-2}	13.037

SCT; Seed Coat Thickness, **WRCS**; Weight Required to Crack Seed, **CS**; Crushing Strength, **PY**; Popping Yield

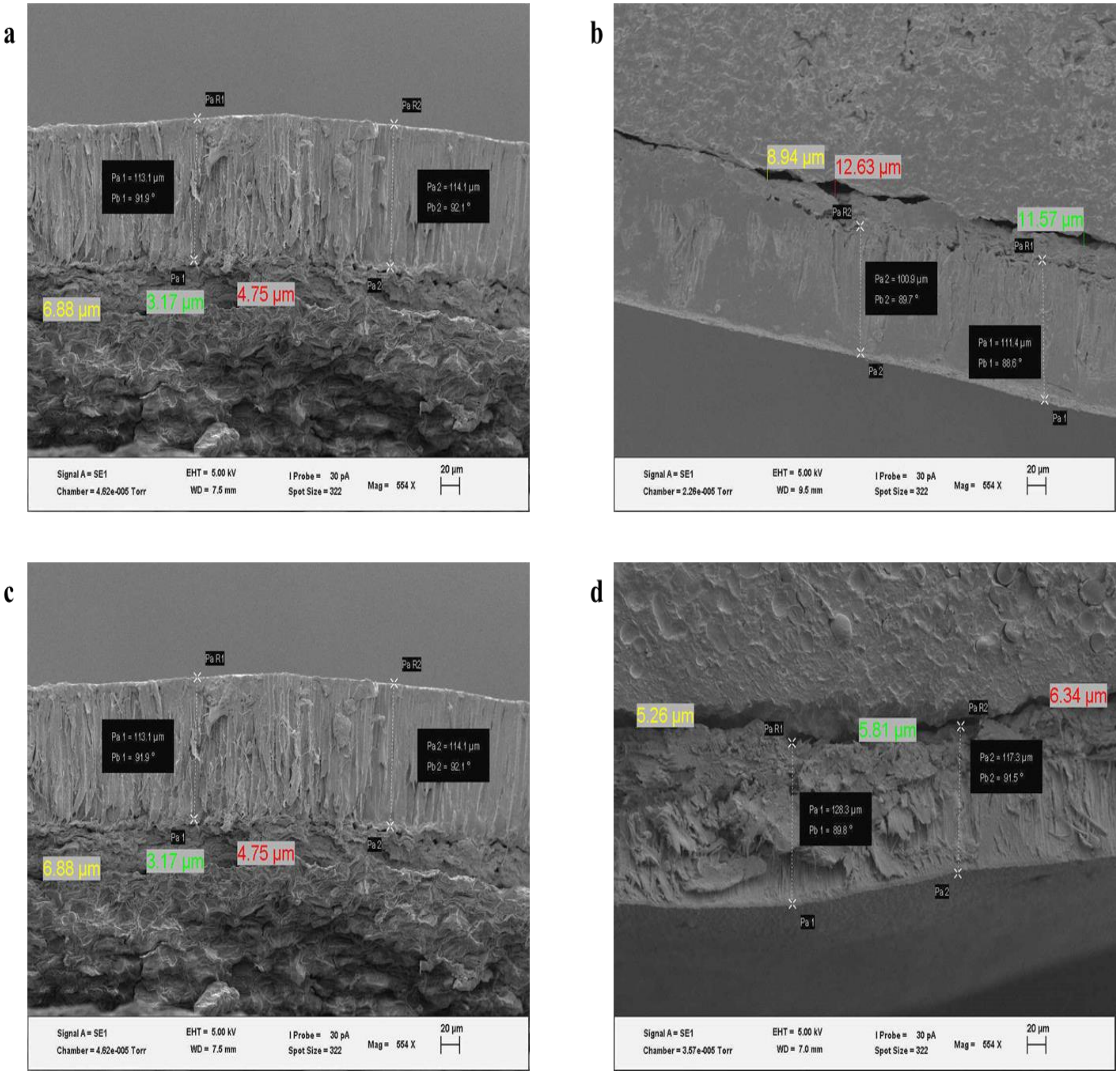


Figure 3.3: Illustration of seed coat thickness measured using a scanning electron microscope among the tested accessions of citron watermelon with varied seed coat colours

3.3.4 Nutrient composition

Significant ($P<0.05$) differences in mineral elements were observed among studied accessions. For Ca, accessions WWM-21, WWM-07 and WWM-24 had higher concentrations (> 5230 mgL^{-1}). Lower Ca concentrations (< 4560 mgL^{-1}) were recorded in WWM-79, WWM-23 and WWM-02. For K, higher concentrations (> 640 mgL^{-1}) were recorded for WWM-02, WWM-45(B) and WWM-35(1). Accessions WWM-21, WWM-45(A) and WWM-35(2) recorded lower K concentrations (< 636 mgL^{-1}). Significantly high Mg concentrations (> 493 mgL^{-1}) were recorded for WWM-76, WWM-05 and WWM-64(2). Lower Mg concentrations (< 487 mgL^{-1}) were recorded for accessions WWM-17(2), WWM-44 and WWM-45(A) (Table 3.4).

Higher Fe concentrations (≥ 70.400 mgL^{-1}) were recorded in WWM-37(2), WWM-68 and WWM-64, while lower concentrations (≤ 65.490 mgL^{-1}) were recorded in WWM-79, WWM-44 and WWM-39. For Cu, WWM-17(2), WWM-44, WWM-08 and WWM-38 recorded higher concentrations (≥ 6.350 mgL^{-1}). Lower Cu concentrations (≤ 5.458 mgL^{-1}) were recorded for WWM-03, WWM-40 and WWM-64 (Table 3.4). Higher Zn concentrations (≥ 101.350 mgL^{-1}) were recorded in WWM-79, WWM-35(2) and WWM-41(A), while lower concentrations (≤ 97.720 mgL^{-1}) were recorded in WWM-50, WWM-09 and WWM-76. Lower Cu concentrations (≤ 13.590 mgL^{-1}) were recorded for accessions WWM-45(B), WWM-66 and WWM-64.

Elemental analysis (C, N and S) differs significantly among landraces ($P < 0.05$). For C, accessions WWM-64, WWM-02, WWM-08 and WWM-15 had higher content (> 53%), while accessions WWM-05, WWM-68 and WWM-42 had lower C content (< 48%). For N, accessions WWM-64, WWM-79 and WWM-68 had higher content (≥ 3.575%), while accessions WWM-07, WWM-45(B) and WWM-05 had lower N content (≤ 2.081%). Higher S (> 3%) content was recorded for accessions WWM-68, WWM-43 and WWM-65, whereas the lowest S content was recorded for WWM-07 (0.094%) (Table 3.4).

Dietary fibre (hemicellulose, cellulose and lignin) varied significantly among the accessions examined. For hemicellulose, WWM-46, WWM-68 and WWM-21 recorded higher values (< 20.5%), while accessions WWM-79, WWM-34 and WWM-35(2) recorded lower hemicellulose content (< 18%). Cellulose was higher (≥ 40.370%) in WWM-46, WWM-15 and WWM-68, and lower (≤ 37.59%) in WWM-79, WWM-34 and WWM-81. For lignin, WWM-65, WWM-68 and WWM-46 had higher content (≥ 30.850%), while WWM-34, WWM-79 and WWM-81 had lower content (≤ 29.340%) (Table 3.4).

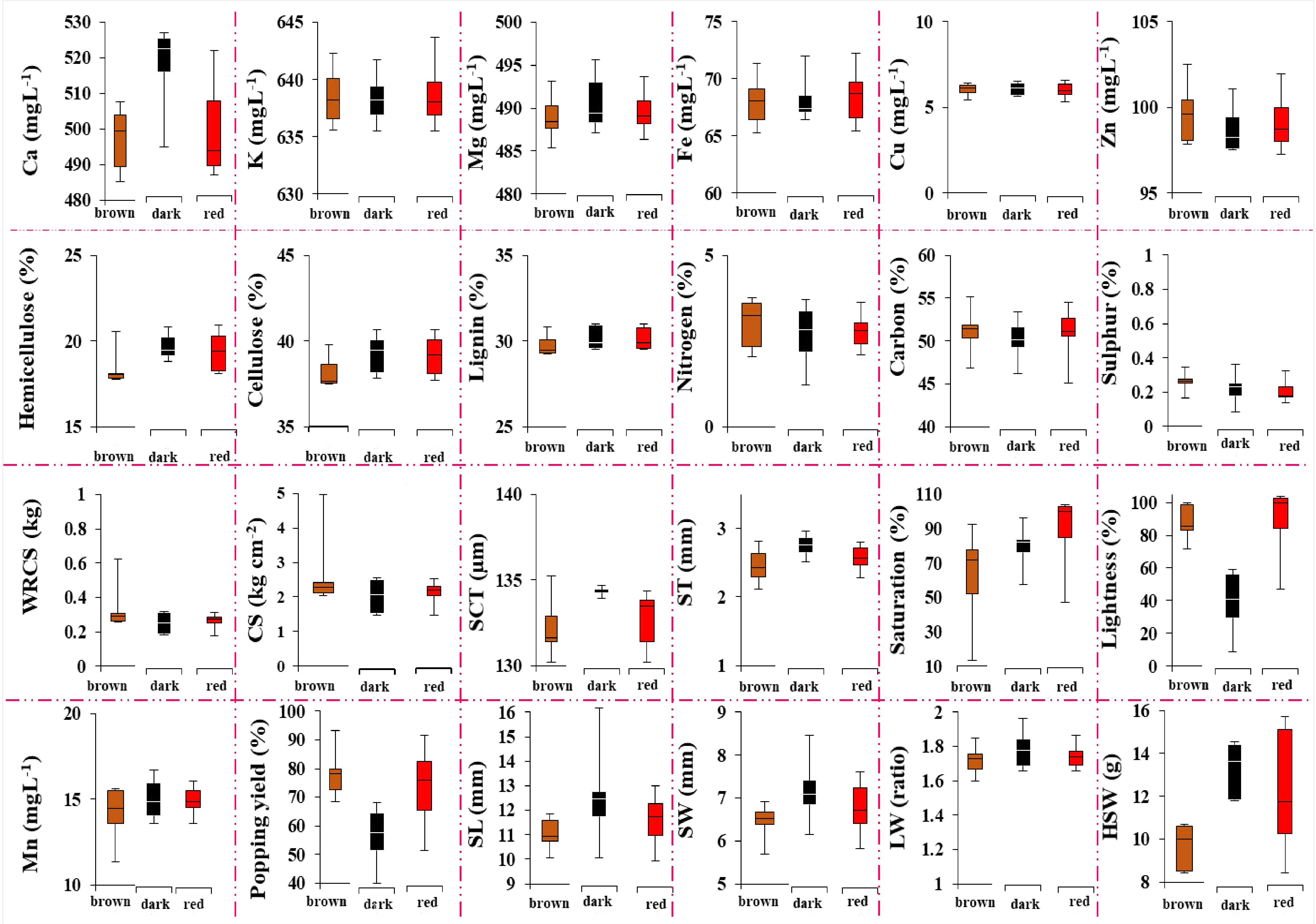


Figure 3.4: Variation in nutritional, physical and mechanical properties of citron watermelon accessions based on seed coat colour

Table 3.4: Mineral elements and dietary fibre contents of 40 citron watermelon accessions with varied seed coat colours

	Mineral elements (mgL^{-1}) [FAAS]							**Elemental Analysis (%) [LECO]**			**Dietary Fiber (%)**		
Genotype	**Ca**	**K**	**Mg**	**Fe**	**Cu**	**Zn**	**Mn**	**N**	**C**	**S**	**Hemicellulose**	**Cellulose**	**Lignin**
WWM-02	498.700^{q}	643.200^{D}	489.100^{q}	68.090^{n}	5.545^{cd}	98.430^{g}	13.590^{c}	2.930^{lmn}	54.080^{u}	0.222^{cdefghi}	18.12^{bc}	39.97^{r}	29.48^{bcde}
WWM-03	485.800^{b}	638.500^{r}	487.600^{hi}	67.590^{l}	5.265^{a}	97.790^{cd}	15.410^{pq}	2.481^{fghi}	50.280^{ghi}	0.181^{bcdef}	18.12^{bc}	40.12^{s}	29.79^{ij}
WWM-05	493.800^{n}	637.90^{m}	493.900^{C}	66.380^{f}	5.847^{ijk}	99.330^{k}	14.070^{g}	2.079^{b}	48.990^{d}	0.234^{defghi}	19.11^{h}	38.19^{h}	29.46^{bc}
WWM-07	524.300^{I}	638.200^{o}	488.300^{m}	67.320^{j}	5.549^{cde}	100.96^{p}	16.030^{w}	1.237^{a}	49.140^{d}	0.094^{a}	19.33^{i}	39.96^{r}	30.82^{o}
WWM-08	501.500^{s}	637.100^{i}	488.600^{o}	69.860^{w}	6.367^{tuv}	97.910^{de}	15.390^{p}	3.179^{nop}	53.650^{t}	0.241^{fghi}	18.27^{de}	37.82^{de}	29.48^{bcde}
WWM-09	515.500^{D}	640.000^{x}	489.400^{s}	67.350^{j}	6.282^{qrst}	97.600^{b}	15.360^{op}	2.683^{ijkl}	52.650^{r}	0.196^{bcdefgh}	19.56^{j}	39.82^{q}	29.67^{h}
WWM-14	487.400^{f}	639.000^{s}	486.500^{d}	65.920^{d}	5.458^{b}	99.360^{k}	15.460^{qr}	3.051^{mno}	50.120^{fg}	0.182^{bcdef}	18.86^{g}	38.12^{gh}	29.52^{def}
WWM-15	489.500^{i}	639.400^{t}	487.500^{g}	66.440^{fg}	5.959^{m}	101.160^{q}	14.740^{jk}	3.142^{mno}	53.460st	0.222^{cdefghi}	19.70^{k}	40.48^{uv}	30.85^{opq}
WWM-16	505.600^{w}	641.200^{z}	490.600^{x}	69.040^{s}	5.704^{fg}	98.130^{f}	15.050^{m}	2.112^{bcd}	50.540^{hij}	0.158^{ab}	19.33^{i}	38.29^{i}	29.58^{g}
WWM-17(2)	499.500^{r}	635.900^{c}	485.400^{a}	70.400^{y}	6.431^{v}	99.630^{l}	13.610^{c}	3.109^{mno}	51.440^{lmn}	0.255^{hi}	18.09^{b}	37.69^{c}	29.45^{b}
WWM-21	525.300^{J}	635.500^{a}	489.000^{p}	67.440^{k}	5.560^{de}	97.720^{bcd}	16.480^{x}	2.239^{bcdef}	50.200^{fgh}	0.174^{bcde}	20.67^{op}	39.97^{r}	29.98^{l}
WWM-23	485.800^{b}	637.200^{j}	490.500^{w}	66.590^{hi}	6.133^{n}	97.750^{bcd}	15.240^{n}	2.515^{fghij}	50.640^{ij}	0.186^{bcdefg}	18.15^{bcd}	38.35^{ij}	29.47^{bcd}
WWM-24	523.300^{H}	636.500^{f}	491.300^{z}	69.350^{u}	6.222^{opq}	99.560^{l}	13.970^{f}	2.887^{klm}	53.060^{s}	0.235^{efghi}	19.44^{ij}	38.95^{l}	30.81^{o}
WWM-28	506.700^{y}	636.600^{f}	488.100^{l}	67.750^{m}	5.857^{ijk}	98.370^{g}	13.690^{d}	2.088^{bc}	50.870^{jk}	0.182^{bcdef}	19.32^{i}	38.38^{j}	30.35^{m}
WWM-33	489.100^{h}	638.300^{p}	489.300^{r}	65.840^{c}	5.911^{klm}	99.050^{j}	14.870^{l}	2.740^{ijkl}	52.380^{qr}	0.257^{hi}	18.10^{bc}	37.77^{d}	30.73^{n}
WWM-34	503.800^{u}	636.900^{h}	487.600^{h}	68.750^{r}	6.306^{qrstu}	98.070^{ef}	15.550^{t}	3.450^{pqr}	46.910^{c}	0.348^{kl}	17.83^{a}	37.52^{ab}	29.32^{a}
WWM-35(1)	515.800^{E}	641.900^{B}	490.300^{v}	65.490^{b}	5.474^{bc}	98.750^{h}	14.530^{i}	3.518^{qrs}	51.750^{mno}	0.246^{ghi}	20.36^{m}	39.49^{p}	30.35^{m}
WWM-35(2)	486.700^{d}	635.600^{b}	491.200^{y}	66.460^{g}	6.147^{no}	102.100^{t}	15.520st	2.355^{cdefg}	51.390^{lm}	0.167^{bc}	17.85^{a}	37.59^{b}	29.34^{a}
WWM-37(2)	520.500^{G}	637.900^{m}	488.400^{n}	71.820^{B}	5.857^{ijk}	98.400^{g}	14.410^{h}	2.931^{lmn}	53.350st	0.195^{bcdefgh}	19.87^{l}	39.94^{r}	29.96^{l}
WWM-38	493.900^{o}	639.500^{u}	490.100^{u}	69.380^{u}	6.350^{stuv}	100.130^{n}	15.650^{u}	2.567^{ghij}	49.870^{ef}	0.186^{bcdefg}	18.31^{e}	37.97^{f}	29.45^{b}
WWM-39	490.400^{j}	637.400^{k}	487.700^{j}	65.460^{b}	6.312^{rstu}	99.45^{kl}	13.870^{e}	2.648^{hijk}	52.130^{opq}	0.171^{bcd}	19.86^{l}	39.13^{n}	29.75^{i}
WWM-40	488.300^{g}	639.700^{v}	487.100^{f}	69.480^{v}	5.400^{b}	101.350^{r}	15.240^{n}	2.910^{klmn}	50.340^{ghi}	0.196^{bcdefgh}	18.23^{cde}	37.82^{de}	29.46^{bc}
WWM-41(A)	486.000^{c}	636.100^{d}	490.500^{w}	66.570^{h}	5.744^{gh}	101.770^{s}	14.550^{i}	2.770^{jkl}	52.180^{pq}	0.181^{bcdef}	20.36^{m}	39.97^{r}	30.82^{op}
WWM-41(B)	486.800^{e}	638.000^{n}	487.700^{i}	70.340^{y}	6.301^{qrstu}	98.970^{ij}	14.680^{j}	2.378^{defgh}	49.950^{efg}	0.168^{bc}	18.49^{f}	38.11^{g}	29.52^{ef}
WWM-44	491.600^{l}	637.700^{l}	485.900^{b}	65.420^{b}	6.381^{uv}	98.470^{g}	13.910^{ef}	2.173^{bcde}	50.870^{jk}	0.175^{bcde}	20.53^{n}	39.52^{p}	29.83^{jk}
WWM-45(A)	488.300^{g}	635.500^{a}	486.100^{c}	69.400^{u}	5.753^{gh}	100.510^{o}	14.750^{k}	2.081^{bc}	50.650^{ij}	0.177^{bcde}	19.86^{l}	40.34^{t}	29.85^{k}
WWM-45(B)	491.400^{k}	642.300^{C}	488.500^{o}	68.060^{n}	6.283^{qrst}	99.640^{l}	11.370^{a}	2.046^{b}	49.560^{e}	0.263^{ij}	18.07^{b}	38.67^{k}	30.38^{m}
WWM-46	511.100^{B}	636.700^{g}	492.500^{A}	70.030^{x}	5.631^{ef}	100.420^{o}	15.510^{rst}	2.392^{efgh}	51.850^{op}	0.274^{ij}	20.82^{q}	40.54^{v}	30.87^{pq}
WWM-47	506.400^{x}	639.500^{u}	487.100^{f}	69.240^{t}	5.824^{hij}	97.79^{bcd}	14.040^{g}	2.086^{bc}	50.890^{jk}	0.146^{ab}	19.53^{j}	39.05^{m}	30.76^{n}
WWM-50	507.300^{z}	636.100^{d}	487.800^{j}	68.570^{q}	6.191^{nop}	97.340^{a}	15.310^{o}	3.477^{qr}	52.370^{qr}	0.282^{ij}	20.34^{m}	39.36^{o}	29.86^{k}
WWM-57	504.000^{v}	638.100^{n}	488.300^{m}	67.460^{k}	6.274^{pqrs}	97.880^{de}	14.880^{l}	3.249^{opq}	51.160^{kl}	0.262^{i}	18.05^{b}	37.70^{c}	30.84^{opq}
WWM-64	507.600^{A}	640.700^{y}	490.000^{t}	71.350^{z}	5.438^{b}	98.380^{g}	13.570^{c}	3.771^{s}	55.150^{v}	0.265^{ij}	18.10^{b}	38.71^{k}	29.47^{bcd}
WWM-64(2)	502.800^{t}	637.100^{i}	493.100^{B}	66.150^{e}	5.775^{ghi}	98.040^{ef}	14.500^{i}	2.280^{bcdef}	51.870^{op}	0.242^{fghi}	20.57^{no}	39.81^{q}	29.95^{l}
WWM-65	492.600^{m}	636.400^{e}	488.300^{m}	69.550^{v}	5.869^{jkl}	98.060^{ef}	14.680^{j}	2.924^{lmn}	45.100^{a}	0.326^{jkl}	20.28^{m}	40.37^{t}	30.88^{q}
WWM-66	518.500^{F}	641.400^{A}	488.000k	66.670^{i}	6.346^{stuv}	97.750^{bcd}	13.480^{b}	3.132^{mno}	49.820^{ef}	0.247^{ghi}	19.05^{h}	38.17^{gh}	29.55^{fg}
WWM-67	496.300^{p}	637.900^{m}	489.400^{s}	68.490^{p}	6.232^{opqr}	99.040^{j}	15.910^{v}	2.951^{lmn}	49.870^{ef}	0.189^{bcdefg}	18.58^{f}	37.76^{cd}	29.52^{ef}
WWM-68	523.300^{H}	637.100^{i}	487.000^{e}	71.670^{A}	6.124^{n}	98.850^{hi}	15.470^{qrs}	3.611^{rs}	46.230^{b}	0.358^{l}	20.72^{pq}	40.46^{u}	30.87^{pq}
WWM-76	513.800^{C}	638.400^{q}	495.100^{D}	67.290^{j}	5.566^{de}	97.620^{bc}	13.880^{e}	3.429^{pqr}	49.990^{fg}	0.265^{ij}	18.76^{g}	37.87^{e}	29.51^{cdef}
WWM-79	485.300^{a}	636.900^{h}	487.700^{j}	65.290^{a}	5.954^{lm}	102.46^{u}	13.860^{e}	3.683^{rs}	50.650^{ij}	0.285^{ijk}	17.81^{a}	37.51^{a}	29.32^{a}
WWM-81	490.300^{j}	639.900^{w}	488.400^{m}	68.320^{o}	5.860^{ijk}	99.850^{m}	15.630^{u}	3.575^{rs}	51.830^{nop}	0.273^{ij}	17.87^{a}	37.54^{ab}	29.33^{a}
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
L.S.D$_{(0.05)}$	0.038	0.032	0.031	0.037	0.042	0.093	0.033	0.136	0.197	0.031	0.064	0.035	0.025

*Means in the same column followed by the same letter are not significantly different, while figures with different footnote are significantly different according to Fisher's test ($P<0.05$) **Ca**; Calcium, **K**; Potassium, **Mg**; Magnesium, **Fe**; Iron, Cu; Copper, Zn; Zinc, Mn; Manganese, **N**; Nitrogen, **C**; Carbon, **S**; Sulphur, **Hem**; Hemicellulose, **Cell**; Cellulose

3.3.5 Seed physical properties, nutritional and biochemical profiles based on seed coat colour

Seed physical properties and nutritional compositions of the assessed citron watermelon accessions grouped based on seed coat colours (brown, dark and red) are presented in Figure 3.4. Seed length, seed width, seed ratio, seed thickness, SCT and HSW were highest in dark seeds. The significantly low hue of 19.040 ° and high saturation of 88.350 % were detected in red seeds. Significantly ($P = 0.013$), higher lightness was observed in brown seeds (87.810%) and red seeds (76.280%), compared to dark seeds (39.790%). Seed popping yield was highest in brown seeds (77.590%) and lowest in dark seeds (57.710%).

Significantly ($P = 0.023$), the weight required to crush the seed and crushing strength were highest in brown seeds (0.321 kg and 2.553 $kgcm^{-2}$) and lowest in dark seeds (0.253 kg and 2.016 $kgcm^{-2}$). A high Ca concentration of 517.225 mgL^{-1} was detected in dark seeds, and a low concentration of 496.757 mgL^{-1} was detected in red seeds. Brown seeds had the highest Cu concentration (6.052 mgL^{-1}), while lowest Cu concentrations were detected in red seeds (5.892 mgL^{-1}). Magnesium was significantly higher in dark seeds (490.250 mgL^{-1}) than in brown (488.911 mgL^{-1}) and red seeds (488.552 mgL^{-1}). For Mn, lowest concentrations were detected in brown seeds (14.277 mgL^{-1}) and highest concentrations in dark seeds (14.843 mgL^{-1}) (Figure 3.4).

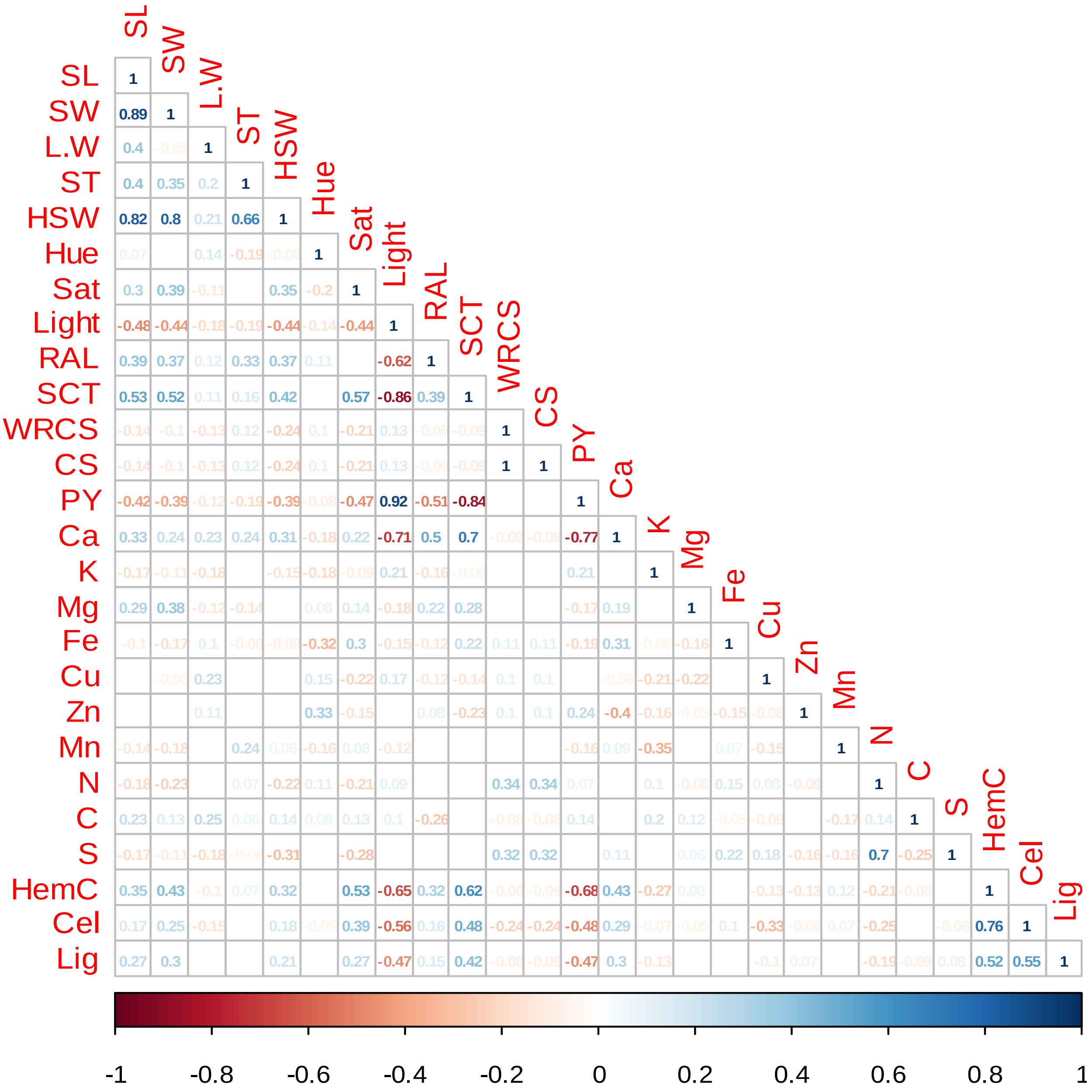
3.3.6 Association between seed physical properties and nutritional compositions

Across all seed coat colours, significant and positive correlations were observed between (i) popping yield and lightness ($r = 0.92$; $p = 0.004$), (ii) calcium and seed coat thickness ($r = 0.70$; $p = 0.04$), (iii) weight required to crack seed and cellulose ($r = 0.48$; $p = 0.007$), (iv) seed coat thickness and hemicellulose ($r = 0.620$; $p = 0.028$) and (v) calcium and hemicellulose ($r = 0.43$; $p = 0.01$). Significant and negative correlations were observed between Ca with (i) seed popping yield ($r = -0.77$; $p = 0.02$), (ii) seed lightness ($r = -0.71$; $p = 0.01$) and (iii) hemicellulose ($r =-0.68$; $p = 0.048$). Seed coat thickness was significantly and negatively correlated with seed lightness and seed popping yield ($r = -0.86$; $p = 0.039$) and ($r = -0.84$; $p = 0.04$) respectively. Hemicellulose content and seed lightness were negatively correlated ($r = -0.65$; $p = 0.02$) (Figure 3.5).

For brown seeds, positive and significant correlations were between (i) Ca and seed coat thickness ($r = 0.86$; $p = 0.003$), (ii) Zn and seed popping yield ($r = 0.82$; $p = 0.014$), (iii) Mg and hemicellulose ($r = 0.68$; $p = 0.022$) and (iv) Mg and cellulose ($r = 0.68$; $p = 0.013$). Strong and negative correlations were observed between (i) Ca and Zn ($r = -0.91$; $p = 0.001$), (ii) Ca and seed popping yield (r = -0.74; p = 0.015) and (iii) seed popping yield and seed coat thickness (r = -0.64; p = 0.018) (Figure 3.6).

For red seeds, strong positive correlations were between seed coat thickness and (i) hemicellulose (r = 0.76; p = 0.016), (ii) Ca (r = 0.55; p = 0.028) and (iii) lignin (r = 0.55; p = 0.024). Seed hemicellulose content in red seeds was also positively correlated with Ca (r = 0.41; p = 0.048). Negative and significant correlations were observed between seed popping yield and (i) seed coat thickness (r = -0.90; p = 0.010), (ii) hemicellulose (r = -0.75; p = 0.039) and (iii) Ca (r = -0.62; p = 0.021) (Figure 3.7).

For dark seeds, strong positive correlation was observed between (i) seed carbon content and HSW (r = 0.83; p = 0.011), (ii) Ca and SCT (r = 0.80; p = 0.005), (iii) Mn and SCT (r = 0.74; p = 0.019) and (iv) Mn and hemicellulose (r = 0.73; p = 0.017) (Figure 3.8). Negative and significant correlations were observed between Mg and (i) cellulose (r = -0.74; p = 0.020), (ii) Ca (r = -0.66; p = 0.032) and (iii) SCT (r = -0.51; p = 0.035). Seed coat thickness and seed sulphur content were also correlated negatively (r = -0.58; p = 0.017) (Figure 3.8).



	SL	SW	L.W	ST	HSW	Hue	Sat	Light	RAL	SCT	WRCS	CS	PY	Ca	K	Mg	Fe	Cu	Zn	Mn	N	C	S	HemC	Cel	Lig
SL	1																									
SW	0.89	1																								
L.W	0.4	-0.05	1																							
ST	0.4	0.35	0.2	1																						
HSW	0.82	0.8	0.21	0.66	1																					
Hue	0.07		0.14	-0.19	-0.06	1																				
Sat	0.3	0.39	-0.11		0.35	-0.2	1																			
Light	-0.48	-0.44	-0.18	-0.19	-0.44	-0.14	-0.44	1																		
RAL	0.39	0.37	0.12	0.33	0.37	0.11		-0.62	1																	
SCT	0.53	0.52	0.11	0.16	0.42		0.57	-0.86	0.39	1																
WRCS	-0.14	-0.1	-0.13	0.12	-0.24	0.1	-0.21	0.13	-0.06	-0.09	1															
CS	-0.14	-0.1	-0.13	0.12	-0.24	0.1	-0.21	0.13	-0.06	-0.09	1	1														
PY	-0.42	-0.39	-0.12	-0.19	-0.39	-0.08	-0.47	0.92	-0.51	-0.84			1													
Ca	0.33	0.24	0.23	0.24	0.31	-0.18	0.22	-0.71	0.5	0.7	-0.09	-0.09	-0.77	1												
K	-0.17	-0.11	-0.18		-0.15	-0.18	-0.09	0.21	-0.16	-0.06			0.21		1											
Mg	0.29	0.38	-0.12	-0.14		0.08	0.14	-0.18	0.22	0.28			-0.17	0.19		1										
Fe	-0.1	-0.17	0.1	-0.08	-0.08	-0.32	0.3	-0.15	-0.12	0.22	0.11	0.11	-0.19	0.31	-0.06	-0.16	1									
Cu		-0.06	0.23			0.15	-0.22	0.17	-0.12	-0.14	0.1	0.1		-0.06	-0.21	-0.22		1								
Zn			0.11			0.33	-0.15		0.08	-0.23	0.1	0.1	0.24	-0.4	-0.16	-0.05	-0.15	-0.08	1							
Mn	-0.14	-0.18		0.24	0.06	-0.16	0.08	-0.12					-0.16	0.09	-0.35		0.07	-0.15		1						
N	-0.18	-0.23		0.07	-0.22	0.11	-0.21	0.09			0.34	0.34	0.07		0.1	-0.08	0.15	0.08	-0.09		1					
C	0.23	0.13	0.25	0.06	0.14	0.08	0.13	0.1	-0.26		-0.08	-0.08	0.14		0.2	0.12	-0.05	-0.09		-0.17	0.14	1				
S	-0.17	-0.11	-0.18	-0.06	-0.31		-0.28				0.32	0.32		0.11		0.06	0.22	0.18	-0.16	-0.16	0.7	-0.25	1			
HemC	0.35	0.43	-0.1	0.07	0.32		0.53	-0.65	0.32	0.62	-0.06	-0.06	-0.68	0.43	-0.27	0.08		-0.13	-0.13	0.12	-0.21	-0.08		1		
Cel	0.17	0.25	-0.15		0.18	-0.05	0.39	-0.56	0.16	0.48	-0.24	-0.24	-0.48	0.29	-0.07	-0.05	0.1	-0.33	-0.06	0.07	-0.25		-0.08	0.76	1	
Lig	0.27	0.3			0.21		0.27	-0.47	0.15	0.42	-0.08	-0.08	-0.47	0.3	-0.13			-0.1	0.07		-0.19	-0.09	0.08	0.52	0.55	1

Figure 3.5: Correlations showing associations of mineral nutritional compositions and seed physical properties across all seed coat colours. **SL**; Seed Length, **SW;** Seed Width, **L.W**; Seed Ratio, **ST**; Seed Thickness, **HSW**; Hundred Seed Weight, Sat; Saturation, **RAL**; Reichs-Ausschuß für Lieferbedingungen und Gütesicherung, **SCT**; Seed Coat Thickness, **WRCS**; Weight Required to Crack Seed, **CS**; Crushing Strength, **PY**; Popping Yield, **Ca**; Calcium, **K**; Potassium, **Mg**; Magnesium, **Fe**; Iron, **Cu**; Copper, **Zn**; Zinc, **Mn**; Manganese, **N**; Percentage Nitrogen, **C**; Percentage Carbon, **S**; Percentage Sulphur, **HemC**; Hemicellulose, **Cel**; Cellulose, **Lig**; Lignin

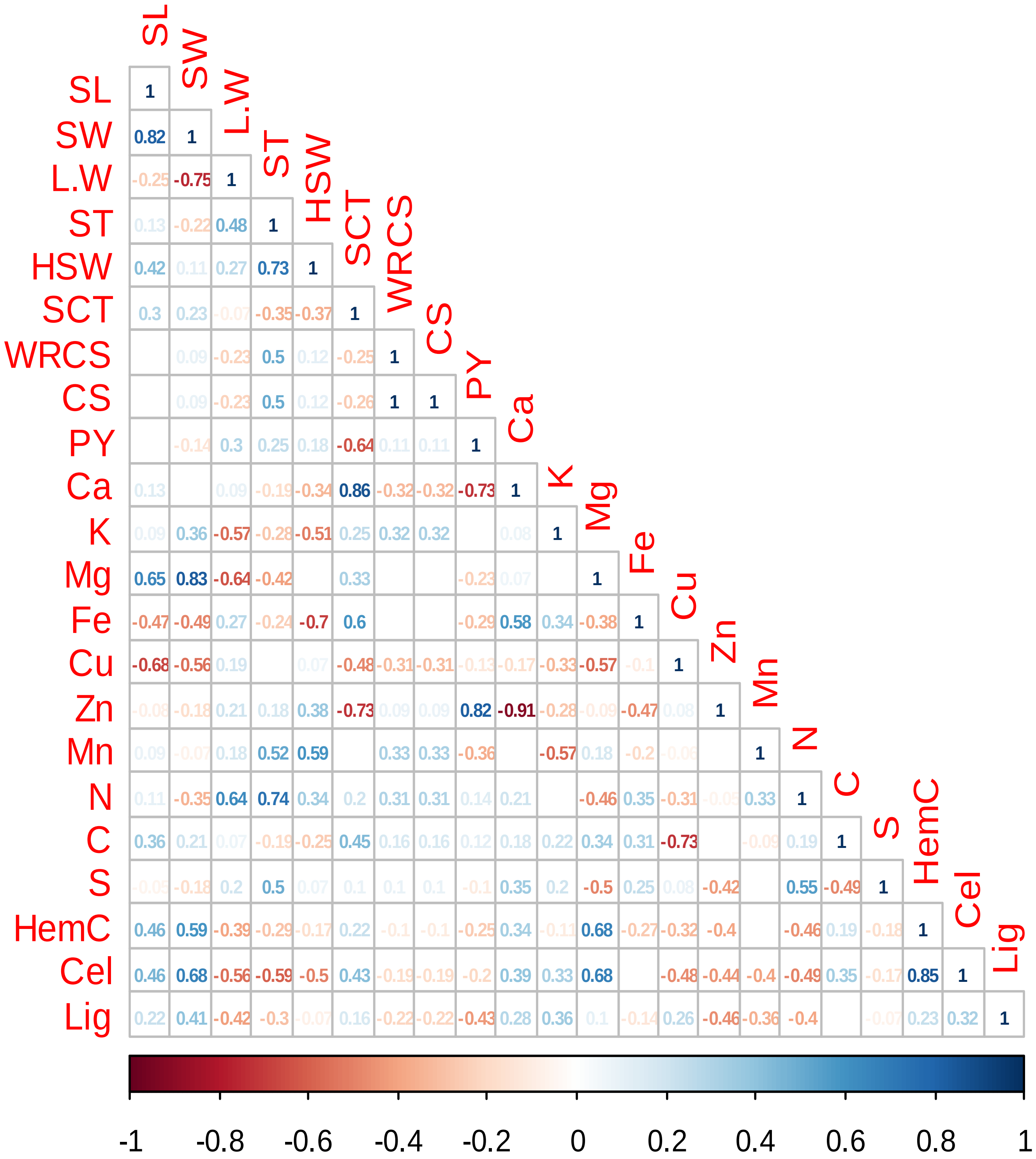
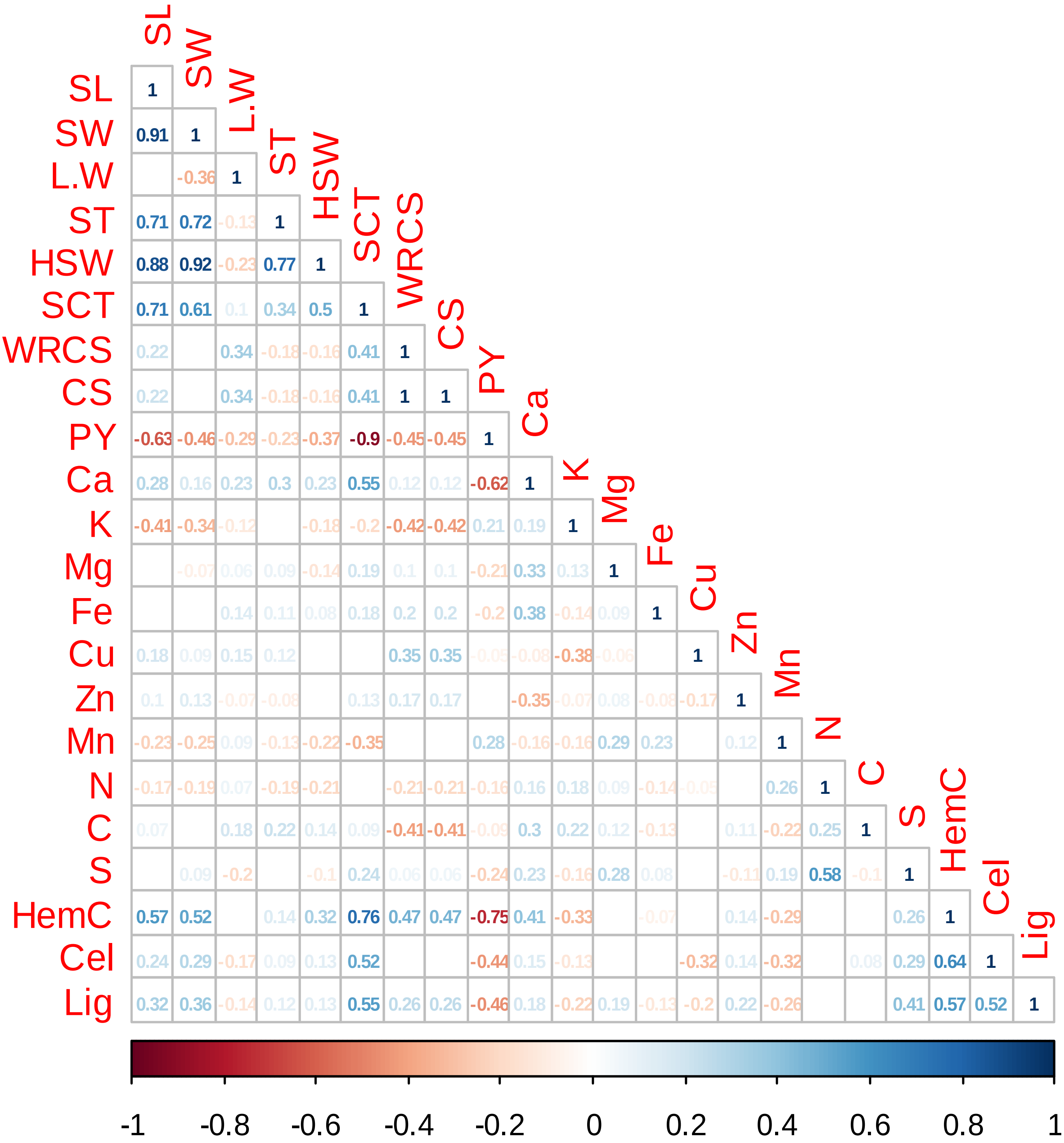
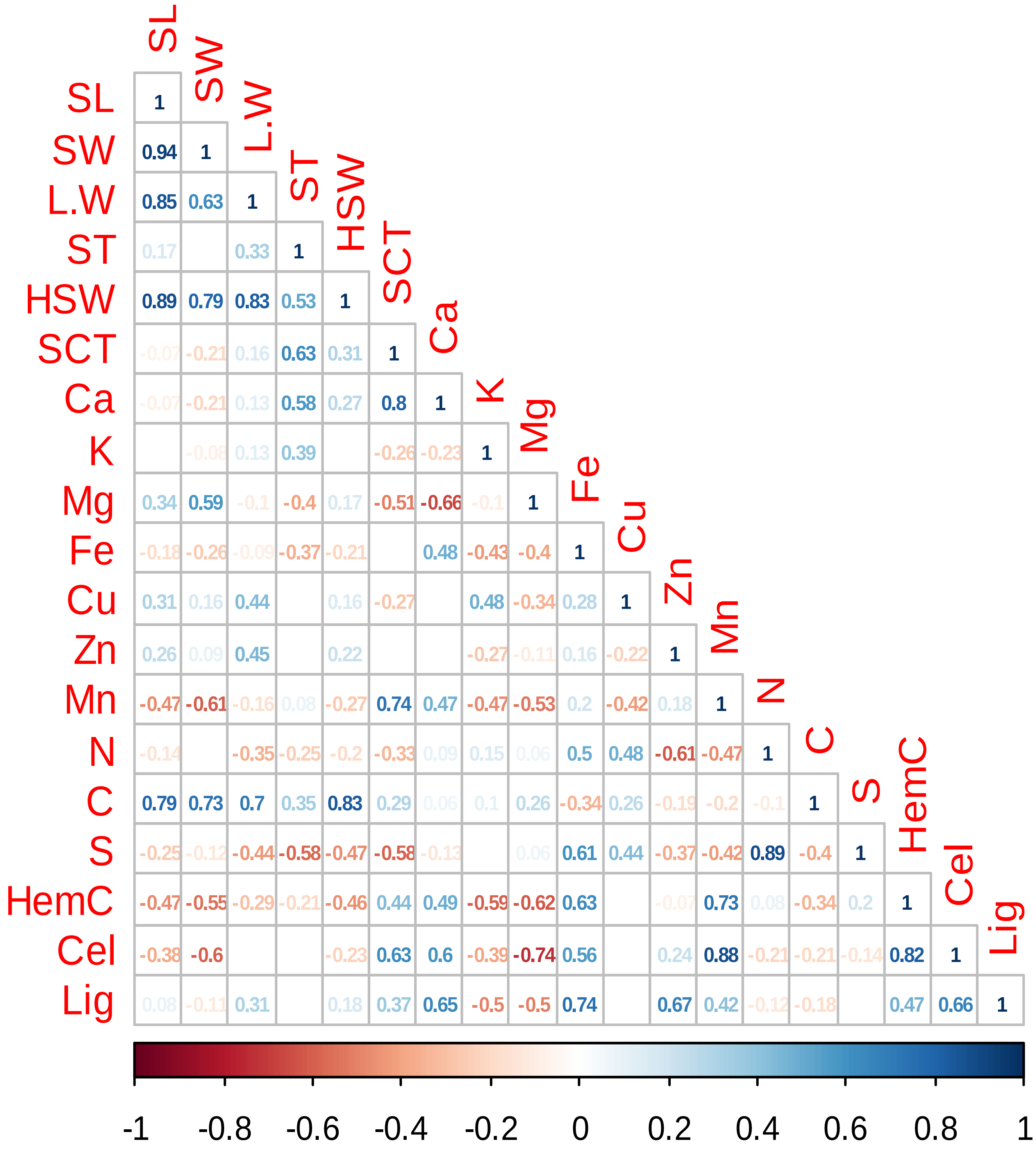


Figure 3.6: Correlations of seed physical, mineral nutrition and biochemical properties for brown seed



	SL	SW	L.W	ST	HSW	SCT	WRCS	CS	PY	Ca	K	Mg	Fe	Cu	Zn	Mn	N	C	S	HemC	Cel	Lig
SL	1																					
SW	0.91	1																				
L.W		-0.36	1																			
ST	0.71	0.72	-0.13	1																		
HSW	0.88	0.92	-0.23	0.77	1																	
SCT	0.71	0.61	0.1	0.34	0.5	1																
WRCS	0.22		0.34	-0.18	-0.16	0.41	1															
CS	0.22		0.34	-0.18	-0.16	0.41	1	1														
PY	-0.63	-0.46	-0.29	-0.23	-0.37	-0.9	-0.45	-0.45	1													
Ca	0.28	0.16	0.23	0.3	0.23	0.55	0.12	0.12	-0.62	1												
K	-0.41	-0.34	-0.12		-0.18	-0.2	-0.42	-0.42	0.21	0.19	1											
Mg		-0.07	0.06	0.09	-0.14	0.19	0.1	0.1	-0.21	0.33	0.13	1										
Fe			0.14	0.11	0.08	0.18	0.2	0.2	-0.2	0.38	-0.14	0.09	1									
Cu	0.18	0.09	0.15	0.12			0.35	0.35	-0.05	-0.08	-0.38	-0.06		1								
Zn	0.1	0.13	-0.07	-0.08		0.13	0.17	0.17		-0.35	-0.07	0.08	-0.08	-0.17	1							
Mn	-0.23	-0.25	0.09	-0.13	-0.22	-0.35			0.28	-0.16	-0.16	0.29	0.23		0.12	1						
N	-0.17	-0.19	0.07	-0.19	-0.21		-0.21	-0.21	-0.16	0.16	0.18	0.09	-0.14	-0.05		0.26	1					
C	0.07		0.18	0.22	0.14	0.09	-0.41	-0.41	-0.09	0.3	0.22	0.12	-0.13		0.11	-0.22	0.25	1				
S		0.09	-0.2		-0.1	0.24	0.06	0.06	-0.24	0.23	-0.16	0.28	0.08		-0.11	0.19	0.58	-0.1	1			
HemC	0.57	0.52		0.14	0.32	0.76	0.47	0.47	-0.75	0.41	-0.33		-0.07		0.14	-0.29			0.26	1		
Cel	0.24	0.29	-0.17	0.09	0.13	0.52			-0.44	0.15	-0.13			-0.32	0.14	-0.32		0.08	0.29	0.64	1	
Lig	0.32	0.36	-0.14	0.12	0.13	0.55	0.26	0.26	-0.46	0.18	-0.22	0.19	-0.13	-0.2	0.22	-0.26			0.41	0.57	0.52	1

Figure 3.7: Correlations of seed physical, mineral nutrition and biochemical properties for red seeds



	SL	SW	L.W	ST	HSW	SCT	Ca	K	Mg	Fe	Cu	Zn	Mn	N	C	S	HemC	Cel	Lig
SL	1																		
SW	0.94	1																	
L.W	0.85	0.63	1																
ST	0.17		0.33	1															
HSW	0.89	0.79	0.83	0.53	1														
SCT	-0.07	-0.21	0.16	0.63	0.31	1													
Ca	-0.07	-0.21	0.13	0.58	0.27	0.8	1												
K		-0.08	0.13	0.39		-0.26	-0.23	1											
Mg	0.34	0.59	-0.1	-0.4	0.17	-0.51	-0.66	-0.1	1										
Fe	-0.18	-0.26	-0.09	-0.37	-0.21		0.48	-0.43	-0.4	1									
Cu	0.31	0.16	0.44		0.16	-0.27		0.48	-0.34	0.28	1								
Zn	0.26	0.09	0.45		0.22			-0.27	-0.11	0.16	-0.22	1							
Mn	-0.47	-0.61	-0.16	0.08	-0.27	0.74	0.47	-0.47	-0.53	0.2	-0.42	0.18	1						
N	-0.14		-0.35	-0.25	-0.2	-0.33	0.09	0.15	0.06	0.5	0.48	-0.61	-0.47	1					
C	0.79	0.73	0.7	0.35	0.83	0.29	0.06	0.1	0.26	-0.34	0.26	-0.19	-0.2	-0.1	1				
S	-0.25	-0.12	-0.44	-0.58	-0.47	-0.58	-0.13		0.06	0.61	0.44	-0.37	-0.42	0.89	-0.4	1			
HemC	-0.47	-0.55	-0.29	-0.21	-0.46	0.44	0.49	-0.59	-0.62	0.63		-0.07	0.73	0.08	-0.34	0.2	1		
Cel	-0.38	-0.6			-0.23	0.63	0.6	-0.39	-0.74	0.56		0.24	0.88	-0.21	-0.21	-0.14	0.82	1	
Lig	0.08	-0.11	0.31		0.18	0.37	0.65	-0.5	-0.5	0.74		0.67	0.42	-0.12	-0.18		0.47	0.66	1

Figure 3.8: Correlations of seed physical, mineral nutrition and biochemical properties for dark seed

3.3.7 Principal component analysis for seed physical properties, nutritional and biochemical compositions

The PCA showing eigenvectors, eigenvalues and percent variance among the seed traits and nutritional compositions are presented in Table 3.5. PCA identified five PC's with eigenvalues ≥ 1, which accounted for 61.29% of the total variation. PC1 accounted for 26.77%

of the total variation and positively correlated to SCT and Ca concentration. PC2 correlated with N, S and Ca and contributed to 12.14% of total variation. PC3 accounted for 8.81% of the total variation and positively correlated with K and C contents. Lignin and K concentrations correlated with PC4 which accounted for 6.90% of the total variation. PC5 accounted for 6.67% of the total variation, positively correlated with saturation, lignin, and S content (Table 3.5).

Table 3.5: Summary of factor loadings, eigenvalue, Kaiser-Meyer-Olkin measure of sampling adequacy, percent and cumulative variation for physical, nutritional and biochemical traits assessed among 40 citron watermelon accessions

Trait	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**KMO**
Lightness	-0.784	-0.145	0.285	-0.229	0.160	0.775
Hue	0.094	0.140	0.025	0.094	-0.276	0.324
Saturation	-0.091	-0.357	0.016	0.068	0.491	0.441
Hemicellulose	0.870	-0.103	-0.089	-0.083	0.217	0.772
Lignin	0.596	0.014	-0.221	0.258	0.479	0.782
Cellulose	0.766	-0.148	-0.086	0.251	0.200	0.705
WRCS	0.690	-0.451	0.245	-0.390	0.005	0.625
C.S	0.690	-0.451	0.245	-0.390	0.005	0.625
P.Y	-0.836	-0.343	0.061	-0.047	0.031	0.777
SCT	0.763	0.301	0.176	0.162	0.038	0.852
Ca	0.672	0.481	0.223	0.073	-0.219	0.753
K	-0.195	0.030	0.600	0.493	-0.093	0.451
Mg	0.228	-0.159	0.496	-0.300	-0.048	0.311
Fe	0.127	0.537	-0.096	0.139	-0.108	0.561
Cu	-0.233	0.306	-0.215	-0.303	0.082	0.432
Zn	-0.233	-0.340	-0.411	0.262	0.277	0.585
Mn	0.186	-0.020	-0.464	-0.271	-0.446	0.374
N%	-0.279	0.609	0.261	-0.171	0.298	0.390
C%	-0.063	-0.209	0.529	0.300	-0.024	0.196
S%	-0.083	0.665	0.131	-0.366	0.480	0.313
Eigenvalue	5.354	2.429	1.761	1.381	1.334	-
Variability (%)	23.771	12.583	8.806	6.903	6.670	-
Cumulative (%)	26.771	38.913	47.719	54.622	61.292	-

***KMO**; Kaiser-Meyer-Olkin measure of sampling adequacy, **WRCS**; Weight Required to Crack Seed, **CS**; Crushing Strength, **PY**; Popping Yield, SCT; Seed Coat Thickness, Ca; Calcium, K; Potassium, **Mg**; Magnesium, **Fe**; Iron, **Cu**; Copper, **Zn**; Zinc, **Mn**; Manganese, **N**; Nitrogen, **C**; Carbon, **S**; Sulphur

The Kaiser Meyer-Olkin measure of sampling adequacy statistic was 0.716 (Table 3.5). Since the value is > 0.7, the data was adequate for PCA. The PC biplot showing correlations between the studied traits with citron watermelon accessions for physical, nutritional and biochemical traits are shown in Figure 3.9. Accessions WWM-33, WWM-38, WWM-40 and WWM-79 grouped based on high popping ability. Accessions WWM-02, WWM-23 and WWM44, are grouped based on high Zn concentrations. WWM-34, WWM-57 and WWM-64 are grouped based on high WRCS, high percentage N and S. Accessions WWM-21, WWM-37(2), WWM-46 and WWM-66 are grouped based on high contents of lignin, cellulose and hemicellulose.

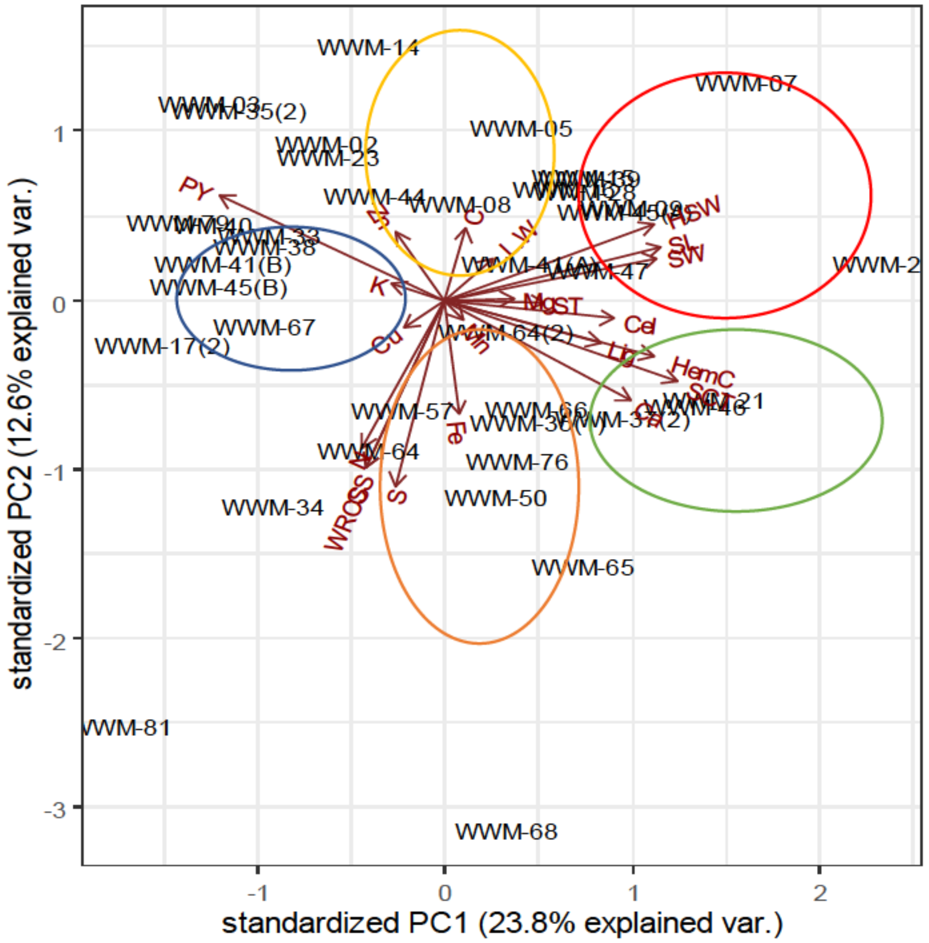


Figure 3.9: Principal component (PC) biplot of PC1 vs PC2 showing relationships for seed physical, nutritional and biochemical compositions among citron watermelon accessions

3.4 Discussion

Roasted citron watermelon seeds serve as a vital nutrient source in many parts of SSA (Mandizvo et al., 2021). Understanding the seed's physical, nutritional and biochemical properties that influence roasting and palatability is vital for developing breeding strategies for variety design in crop improvement programs. The present study determined associations between physical, nutritional and biochemical properties in relation to seed coat colour in citron watermelon. Distinctive differences were observed for seed physical properties (seed thickness, HSW, seed length, seed width, SCT, seed crushing strength and WRCS), nutritional (Ca, Mg, Mn, Cu, Fe, Zn, K, N and P) and biochemical (cellulose, hemicellulose and lignin) compositions among the studied accessions which were closely associated with seed coat colours. This will allow selecting suitable accessions for food, feed, processing, value-addition with desired seed coat colours, breeding nutritionally improved genotypes with farmer-and-consumer preferences.

Chewability of citron watermelon seed influences palatability which can be influenced by physical properties, including seed coat thickness. Growers widely prefer red and white seed types for preparing roasted seed snacks. In the present study, accessions with red-white seed coats such as WWM-02, WWM-03, WWM-14, WWM-16 and WWM-23 possessed soft-to-medium hard seed coats (~ 2.520 kg cm^{-2}). According to the current study, farmers could consciously select accessions with red-white and white seed coats for roasting. On the contrary, accessions with dark red and maroon seed types (WWM-39, WWM-46, WWM-47 and WWM-50) had thicker seed coats (> 133.60 µm), higher WRCS (> 0.285 kg) and CS (> 2.265 kgcm^{-2}) compared to red-white-and white seeds (Table 3.3). Accessions with dark brown or black seeds (WWM-05, WWM-07, WWM-09, WWM-21 and WWM-66) had thick seed coats (> 133.90 µm), WRCS (> 0.274 kg) and CS (> 2.178 kgcm^{-2}) (Table 3.3). This indicated that dark-coloured seed types are unsuitable for roasting. This is partly explained by a low frequency of these types in the present study (Figure 3.2). Furthermore, the present study supported indigenous knowledge that dark-red, dark-brown and black seeds are relatively difficult to chew and unsuitable for use as seed snacks (Peter, 2008).

Roasting causes popping, whereby the seed cracks and releases oil, which improves flavour and palatability. Red-white seeded accessions such as WWM-02, WWM-03, WWM-14, WWM-16, WWM-23 and WWM-28 possess excellent popping ability (popping yield > 70%). Contrastingly, dark and brown seeded accessions such as WWM-05, WWM-07, WWM-09, WWM-66 and WWM-68 were relatively low popping ability (< 50%). Non-popping seeds are prone to burning during high-temperature roasting (Devi et al., 2020). The present study indicated that red seeds have desirable physical properties for preparing roasted seed snacks than dark black and brown seeds.

Seeds may be a valuable source of essential mineral elements for improving human health. Mandizvo and Odindo (2020) reported that, in seeds, phytates are synthesized to store phosphates and divalent cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+} and Cu^{2+}) which are released when the phytates are broken down during germination, cooking or sprouting. The present study revealed that citron watermelon accessions with varied seed coat colours were vital sources of essential mineral elements including Ca, K, Mg, Mn, Cu, Zn and Fe. Seed Ca content for the studied accessions ranged between 485.3 and 525.3 mgL$^{-1,}$ which were comparable to Ca concentration of 490 mgL^{-1} reported by Fokou et al. (2004) in seeds of egusi melon (*Cucumeropsis mannii*). Calcium is required in the body to prevent osteoporosis (a disease in which bones become fragile and prone to fracture) (Wawrzyniak and Suliburska, 2021). Accessions such as WWM-21, WWM-07, and WWM-24 were good sources of Ca for supplementation to prevent bone diseases.

Dark and brown seeds had high Mg concentrations, while dark and red seeds had high K concentrations (Table 3.4). Accessions with varied seed coat colours could improve nutrient intake and develop value-added products based on the intended use. Micronutrient deficiencies, mainly Zn and Fe, are common in African and Asian countries, affecting more than half of the human population (Siwela et al., 2020). In addition, micronutrient deficiency (hidden hunger) causes malnutrition in children (Vassilakou, 2021). The present study revealed that citron watermelon seeds are rich in Fe and Zn, with a high concentration of Cu and Zn found in brown seeds. In contrast, red seeds are high in Mn and Fe (Table 3.4). Therefore, the consumption of citron watermelon seed could be a valuable strategy to reduce micronutrient-related illnesses in Africa and Asia. Zinc and Fe-biofortified genotypes could also be developed in watermelon breeding programmes using some genotypes identified in the present study.

Lignin, cellulose, and hemicellulose are essential seed coat structural components that may influence citron watermelon seeds' palatability and roasting quality. The present study revealed that red seeded accessions (WWM-46, WWM-15 WWM-65 and LIMP-45(A)) had high hemicellulose (> 19.70%), cellulose (> 40.34%) and lignin (> 29.85%) contents, which were higher than brown seeded accessions (Table 3.4). Also, dark seeded accessions (WWM-07, WWM-21, WWM-24 and WWM-68) possessed significantly higher content of hemicellulose (> 19.33%), cellulose (> 38.95%) and lignin (> 29.98%) contents compared to brown seeds. This partly explains their thick seed coats and slightly higher force required to crush the seed when consuming the roasted seed. Shen et al. (2019) reported that a high density of lignin increased the hardness of *Cucurbita moschata* seeds; hence the seeds are hard to chew.

The negative correlations (Figure 3.5) between popping yield and Ca (r = -0.77), hemicellulose (r = -0.68), cellulose (r = -0.48) and lignin (r= -0.47) indicated physiological roles of these structural compounds in increasing cell stability and rigidity of the seeds thus reducing popping ability during roasting. Calcium strengthens the cell walls by bridging cellulosic microfibrils embedded in a matrix of non-cellulosic polysaccharides and lignin (Chiang et al., 1994). The positive associations between Ca and seed coat thickness for dark (r = 0.8), red (r = 0.55) and brown (r = 0.86) demonstrated the calcium-binding ability of fibres (James et al., 1978; Weber et al., 2002).

Cucurbit seed coats are composed of (i) a deposit of waxy material interfused with lipoidal substance (β-sitosterol), (ii) a sub subjacent encrustation of hemicellulose-cellulose complex and (iii) a layer of palisade cells in which the secondary walls are impregnated with arabinan and the lumen containing tannins and phenolic compounds (Mortensen et al., 2021). The positive correlations between hemicellulose and seed coat thickness for red (r = 0.796), dark (r = 0.494) and brown (r = 0.22) seeds, respectively, implied increased deposition of hemicellulose on citron watermelon seed coat. Also, the present results suggested that hemicellulose deposition was higher in red seeds than dark and brown seeds. The positive correlations between seed coat thickness with cellulose were higher for dark seeds (r = 0.63), red (r = 0.52) and brown (r = 0.43) seeds, whereas associations between seed coat thickness with lignin were higher for red (r = 0.55), dark (r = 0.37) and brown (r = 0.16), respectively. Increase concentrations of Ca was found to reduce Zn content (r = -0.91) but increase Fe content (r = 0.58) of brown seed, whereas increased Ca content resulted in moderate increase in Fe content for red and dark seeds (r = 0.38 and r = 0.48, respectively) but slightly reduced contents of Zn for red and dark seeds (r = -0.35 and r = -0.27, respectively) (Figure 3.6, 3.7 and Table 3.8). These associations have implications for developing genetically superior genotypes of citron watermelon for food and feed and other useful applications.

3.5 Conclusion

In conclusion, seed coat colour is an important phenotypic trait to consider when selecting citron watermelon for roasting and processing other value-added products. Brown and red-coloured seeds have a higher popping yield than dark-coloured seeds with poor popping ability and are prone to burning during roasting. The popping ability of the seed is associated with increased calcium concentration. In contrast, seed coat thickness was closely related to hemicellulose contents and cellulose across all seed coat colours. High hemicellulose, cellulose and lignin contents were found in dark and red seeds associated with thick seed coats and increased chewing strength than white seeds. From a nutritional perspective, dark and red seeds were good sources of Cu, Zn, nitrogen and sulfur than brown seeds. Dark and brown seeds were good Mg sources, whereas dark and red seeds were vital sources of potassium. All seed types were high in zinc and iron. Overall, seed coat colour was closely related to seed physical properties, nutrient and biochemical composition, and the quality of citron watermelon seed. The present findings will guide breeding strategies to develop nutritionally improved genotypes of citron watermelon for food and feed and develop value-added nutraceuticals.

References

Adeyeye S.A.O., Bolaji Olushola T., Abegunde T.A., Adebayo-Oyetoro A.O., Tiamiyu H.K., Idowu-Adebayo F. (2019) Evaluation of nutritional composition, physico-chemical and sensory properties of '*Robo*' (a Nigerian traditional snack) produced from watermelon (*Citrullus lanatus* (Thunb.) seeds. Food Research 4:216-223. DOI: 10.26656/fr.2017.4(1).230.

Bultosa G., Molapisi M., Tselaesele N., Kobue-Lekalake R., Desse Haki G., Makhabu S., Sekwati-Monang B., Seifu E., P. Nthoiwa G. (2020) Plant-based traditional foods and beverages of Ramotswa Village, Botswana. Journal of Ethnic Foods 7. DOI: 10.1186/s42779-019-0041-3.

Chacha J.S., Laswai H.S. (2020) Micronutrients Potential of Underutilized Vegetables and Their Role in Fighting Hidden Hunger. Int J Food Sci 2020:9408315. DOI: 10.1155/2020/9408315.

Chiang W.D., Thomas R.L., Kunkel M.E. (1994) Calcium binding by cellulose and lignin. Food Chemistry 50:191-196. DOI: 10.1016/0308-8146(94)90119-8.

Devi M., Sharma K., Jha S., Arora S., Patel S., Kumar Y., Vishwakarma R. (2020) Effect of popping on physicochemical, technological, antioxidant, and microstructural properties of makhana seed. Journal of Food Processing and Preservation 44. DOI: 10.1111/jfpp.14787.

Fokou E., Achu M., Tchounguep F. (2004) Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. African Journal of Food, Agriculture, Nutrition and Development 4.

Haque M.A., Islam M.P., Hussain M.D., Khan F. (2010) Physical, mechanical properties and oil content of selected indigenous seeds available for biodiesel production in Bangladesh. Agricultural Engineering International: CIGR Journal.

Hollmann J., Themeier H., Neese U., Lindhauer M.G. (2013) Dietary fibre fractions in cereal foods measured by a new integrated AOAC method. Food Chem 140:586-9. DOI: 10.1016/j.foodchem.2012.12.005.

Huang L., Bell R.W., Dell B., Woodward J. (2007) Rapid Nitric Acid Digestion of Plant Material with an Open-Vessel Microwave System. Communications in Soil Science and Plant Analysis 35:427-440. DOI: 10.1081/css-120029723.

James W.P.T., Branch W.J., Southgate D.A.T. (1978) Calcium Binding by Dietary Fibre. The Lancet 311:638-639. DOI: 10.1016/s0140-6736(78)91141-8.

Jarret R.L., Levy I.J. (2012) Oil and fatty acid contents in seed of *Citrullus lanatus* Schrad. J Agric Food Chem 60:5199-204. DOI: 10.1021/jf300046f.

Joshi D.C., Das S.K., Mukherjee R.K. (1993) Physical Properties of Pumpkin Seeds. Journal of Agricultural Engineering Research 54:219-229. DOI: 10.1006/jaer.1993.1016.

Mabaleha M.B., Mitei Y.C., Yeboah S.O. (2006) A Comparative Study of the Properties of Selected Melon Seed Oils as Potential Candidates for Development into Commercial Edible Vegetable Oils. Journal of the American Oil Chemists' Society 84:31-36. DOI: 10.1007/s11746-006-1003-7.

Mandizvo T., Odindo A.O. (2019a) Seed coat structural and imbibitional characteristics of dark and light coloured Bambara groundnut (*Vigna subterranea* L.) landraces. Heliyon 5:e01249. DOI: 10.1016/j.heliyon.2019.e01249.

Mandizvo T., Odindo A.O. (2019b) Seed mineral reserves and vigour of Bambara groundnut (*Vigna subterranea* L.) landraces differing in seed coat colour. Heliyon 5:e01635. DOI: 10.1016/j.heliyon.2019.e01635.

Mandizvo T., Odindo A.O. (2020) Spectrophotometric quantification of phytic acid during embryogenesis in bambara groundnut (*Vigna subterranea* L.) through phosphomolybdenum complex formation. Emirates Journal of Food and Agriculture:778-785. DOI: 10.9755/ejfa.2020.v32.i11.2178.

Mandizvo T., Odindo A.O., Mashilo J. (2021) Citron Watermelon Potential to Improve Crop Diversification and Reduce Negative Impacts of Climate Change. Sustainability 13. DOI: 10.3390/su13042269.

Mortensen A.K., Gislum R., Jørgensen J.R., Boelt B. (2021) The Use of Multispectral Imaging and Single Seed and Bulk Near-Infrared Spectroscopy to Characterize Seed Covering Structures: Methods and Applications in Seed Testing and Research. Agriculture 11. DOI: 10.3390/agriculture11040301.

Nguyen H.T., Sárosi S.T., Llorens-Molina J.A., Ladányi M., Zámborine-Németh É. (2018) Compositional variability in essential oils of twelve wormwood (*Artemisia absinthium* L.)

accessions grown in the same environment. Journal of Essential Oil Research 30:421-430. DOI: 10.1080/10412905.2018.1496856.

Nkoana D.K., Mashilo J., Shimelis H., Ngwepe R.M. (2021) Nutritional, phytochemical compositions and natural therapeutic values of citron watermelon (*Citrullus lanatus* var. *citroides*): A Review. South African Journal of Botany. DOI: 10.1016/j.sajb.2020.12.008.

Peter K. (2008) Underutilized and Underexploited Horticultural Crops: Vol. 04 New India Publishing.

Shen Q., Wu L., Zhao X., Li Y. (2019) Comparative analysis of electron microscopic structure of seed testa, lignin and biosynthesis-related enzyme activities in hulled and hull-less seeds of *Cucurbita moschata*. Scientia Horticulturae 245:137-143. DOI: 10.1016/j.scienta.2018.10.005.

Singh N.P., Matta N.K. (2010) Levels of seed proteins in Citrullus and Praecitrullus accessions. Plant Systematics and Evolution 290:47-56. DOI: 10.1007/s00606-010-0347-5.

Siwela M., Pillay K., Govender L., Lottering S., Mudau F.N., Modi A.T., Mabhaudhi T. (2020) Biofortified Crops for Combating Hidden Hunger in South Africa: Availability, Acceptability, Micronutrient Retention and Bioavailability. Foods 9. DOI: 10.3390/foods9060815.

Vassilakou T. (2021) Childhood Malnutrition: Time for Action. Children (Basel) 8. DOI: 10.3390/children8020103.

Wawrzyniak N., Suliburska J. (2021) Nutritional and health factors affecting the bioavailability of calcium: a narrative review. Nutr Rev. DOI: 10.1093/nutrit/nuaa138.

Weber C.W., Kohlhepp E.A., Idouraine A., Ochoa L.J. (2002) Binding capacity of 18 fiber sources for calcium. Journal of Agricultural and Food Chemistry 41:1931-1935. DOI: 10.1021/jf00035a023.

Wu Y., Shen Y.B. (2020) Seed Coat Structural and Permeability Properties of Tilia miqueliana Seeds. Journal of Plant Growth Regulation. DOI: 10.1007/s00344-020-10179-0.

Zia S., Khan M.R., Shabbir M.A., Aadil R.M. (2021) An update on functional, nutraceutical and industrial applications of watermelon by-products: A comprehensive review. Trends in Food Science & Technology 114:275-291. DOI: 10.1016/j.tifs.2021.05.039.

Chapter 4: Drought tolerance assessment of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) accessions based on morphological and physiological traits

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Abstract

Long-term cultivation of citron watermelon under water-constrained environments in sub-Saharan Africa resulted in the selection and domestication of highly tolerant genotypes. However, information on the magnitude of variation for drought tolerance in citron watermelon is limited for the effective selection of suitable genotypes for breeding. The objective of this study was to determine variation for drought tolerance among South African citron watermelon landrace accessions for selection and use as genetic stock for drought-tolerance breeding in this crop and closely-related cucurbit crops. Forty genetically differentiated citron watermelon accessions were grown under non-stress (NS) and drought-stress (DS) conditions under glasshouse environment. Data of physiological (i.e., leaf gas exchange and chlorophyll fluorescence parameters) and morphological traits (i.e., shoot and root system architecture traits, and fruit yield) were collected and subjected to various parametric statistical analyses. The accessions varied significantly for assessed traits under both NS and DS conditions which aided classification into five groups, namely; A (highly drought-tolerant), B (drought-tolerant), C (moderate drought-tolerant), D (drought-sensitive) and E (highly drought-sensitive). Drought-tolerant genotypes produced more fruit yield with less water compared with drought-sensitive genotypes. Several physiological and morphological parameters correlated with fruit yield under DS condition namely: instantaneous water-use efficiency (r = 0.97), leaf dry weight (r = 0.77), total root length (r =0.46) and root dry weight (r = 0.48). The following accessions, namely: WWM-46, WWM-68, WWM-41(A), WWM-15, WWM-64, WWM-57, WWM-47, WWM-37(2), WWM-79, WWM-05 and WWM-50) were identified as highly drought-tolerant and recommended for drought-tolerance breeding in this crop or related cucurbit crops such as sweet dessert watermelon.

Keywords: Abiotic stress, Chlorophyll fluorescence, Drought tolerance indices, Leaf gas exchange, Root phenes

4.1 Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) is an important cucurbit crop widely cultivated in arid and semi-arid environments of sub-Saharan Africa (SSA) for food and feed (Mandizvo et al., 2022; Mashilo et al., 2021; Ngwepe et al., 2021a). In the region, the primary uses of the crop include the cooking of tender leaves as a vegetable, vines as fodder for animals, use of seed as snack and feed for birds (i.e., chickens), and mature fruits for cooking and salad making, and for use as animal feed (Mandizvo et al., 2021; Ngwepe et al., 2019; Nkoana et al., 2021). The crop thrives under insufficient and untimely precipitation attributed to its heat and drought-tolerance ability due to its efficient drought avoidance, tolerance and escape mechanisms (Guzzon et al., 2017; Mo et al., 2015), strategies that enable the crop to produce sufficient above-ground biomass to support fruit and seed yield development under water deficit conditions. Drought tolerance mechanisms in citron watermelon included changes in expression of genes involved in oxidative stress (e.g., glutathione peroxidase, glucose-6-phosphate-dehydrogenase, and ascorbate peroxidase) and accumulation of osmolytes such as citrulline, glutamate and arginine (Akashi et al., 2002; Takahara et al., 2005a; Takahara et al., 2006). Drought avoidance in this crop is associated with reduced leaf transpiration rates and water loss, enhanced water uptake by the roots, and early flowering to avoid severe drought stress (Hakki et al., 2016; Kajikawa et al., 2010; Liu and Latimer, 1995). Its highly-drought tolerant nature has rendered the crop as a potential rootstock to improve fruit yield and quality of sweet watermelon (*Citrullus lanatus* var. *lanatus*) especially for water-limited environments (Edelstein et al., 2014; García-Mendívil et al., 2019; Kombo and Sari, 2019; Pal et al., 2020; Seymen et al., 2021; Yavuz et al., 2020).

Sweet watermelon is an important crop widely grown for its nutritious fruit in many countries. However, the crop is susceptible to various biotic (i.e., pest, bacterial and viral diseases) and abiotic stress factors including heat and drought stress (Malambane et al., 2021; Mo et al., 2016). This is attributed to the continuous selection of the crop for desirable horticultural traits which resulted in limited genetic variation for breeding (Minsart et al., 2010; Singh et al., 2017; Solmaz et al., 2010; Stone et al., 2019; Yang et al., 2016; Zhang et al., 2016). Compared to sweet dessert watermelon, citron watermelon exhibits high levels of drought adaptation, making it an attractive source of useful genetic traits for improving drought tolerance in the cultivated sweet dessert watermelon (Malambane et al., 2021). However, incorporating valuable genes for improving drought tolerance will require knowledge on the genetic variability for drought tolerance among citron watermelon genetic resources, of which currently there is scant information. Also, the development of citron watermelon germplasm with enhanced levels of drought tolerance is essential for sustainable production of the crop in SSA amidst the increased occurrences of drought episodes.

Physiological responses that confer drought tolerance in citron watermelon have been reported (Akashi et al., 2001a; Malambane et al., 2018; Mo et al., 2015; Nanasato et al., 2005a; Yokota et al., 2002a; Zhang et al., 2011a). These include the biosynthesis of amino acids such as citrulline (Akashi et al., 2001a; Song et al., 2020; Yokota et al., 2002a) and an efficient photosynthesis and photoprotection system (Akashi et al., 2001b; Nanasato et al., 2005b; Takahara et al., 2005b; Yokota et al., 2002b; Yoshimura et al., 2008; Zhang et al., 2011a). Also, morphological responses associated with drought adaptation of citron watermelon have been reported (Guzzon et al., 2017). A well-developed root system has been implicated in drought tolerance and high biomass production and fruit yield in citron watermelon, primarily due to the ability of the crop to extract more water from the soil and transport it to aboveground parts, contributing to better physiological functioning (Bikdeloo et al., 2021; Guzzon et al., 2017).

Developing an efficient screening technique is crucial in the selection and breeding of citron watermelon genotypes with high levels of drought tolerance. Traits that allow biomass production under drought conditions must be identified in early breeding programs to establish effective selection criteria. The value of selecting a trait depends on its variation and correlation with yield under drought conditions (Katuuramu et al., 2020). Agronomic traits and evaluations of genotypes for high yield potential or stable performance under different drought stress treatments are the starting points for drought tolerance breeding (Patel et al., 2019). Therefore, based on yield loss under drought compared to optimal conditions, drought indices such as stress susceptibility index (SSI) (Ayed et al., 2021), yield stability index (YSI) (Sánchez-Virosta et al., 2021), geometric mean productivity (GMP) (Grzesiak et al., 2018) and drought resistance index (DRI) (Bhusal et al., 2021) have been used to screen and identify drought tolerant genotypes.

Physiological traits such as stomatal conductance, transpiration rate, photosynthetic efficiency and protection mechanisms (e.g., photochemical and non-photochemical quenching) have been used to indicate drought tolerance in cucurbits (Mashilo et al., 2018a; Mashilo et al., 2017a; Mashilo et al., 2018b). Drought tolerance assessment of germplasm resources employing morphological and physiological traits combined with yield-based selection indices could increase the efficiency of screening and identification of drought-tolerant genotypes.

For effective use of citron watermelon genetic resources for abiotic stress tolerance breeding in sweet watermelon, there is a need to assess the extent of genotypic variation for drought tolerance in this crop. These will allow the identification and ultimate selection of potential sources of useful genes associated with drought adaptation for breeding. In many parts of South Africa, landrace accessions of citron watermelon are grown primarily in rural communities under rain-fed conditions. The germplasm is continuously subjected to unfavourable weather conditions including drought and heat stress. Under these conditions, local farmers have continuously selected suitable varieties for cultivation for various uses including for vegetable, fodder, and fruit and seed consumption. It is possible that the continuous selection of the harsh under harsh environmental conditions could have led in the development of drought-tolerant genotypes. Therefore, the germplasm requires rigorous evaluation for drought adaptive traits to guide strategic selection and conservation of the crop for future breeding. Therefore, the objective of this study was to determine variation for drought tolerance among South African citron watermelon landrace accessions for selection and use as genetic stock for drought-tolerance breeding in this crop and closely-related cucurbit crops such as sweet watermelon.

4.2 Materials and methods

4.2.1 Plant material

Forty citron watermelon landrace accessions provided by the Limpopo Department of Agriculture and Rural Development; South Africa were used for the study. Initially, the accessions were collected from different villages and districts (i.e., Waterberg, Sekhukhune and Capricorn) of the Limpopo Province. The selected accessions are widely grown by small-holder farmers under rain-fed conditions and are often subjected to prolonged heat and drought stress. Also, the accessions were selected for the present study being morphologically and genetically divergent (Mashilo et al., 2017b; Ngwepe et al., 2021b). Seeds of the accessions were bulked under field condition at Towoomba Research Station during the 2017-18 growing season. The code of accessions, village and district of collection, and associated leaf, fruit and seed traits are presented in Table 4.1.

Table 4.1: Information on source of seed, leaf architecture, fruit shape and seed coat colour of citron watermelon accessions used in the study

Accession code	**District**	**Village**	**Latitude and Longitude coordinates**	**Leaf shape**	**Fruit shape**	**Seed coat colour**
WWM-02	Capricorn	Kgohloane	23°47'11.5"**S** 29°21'56.4"**E**	Lobed	Round	Vermillion
WWM-03	Capricorn	Kgohloane	23°47'11.5"S 29°21'56.4"E	Lobed	Elliptic	Bright red orange
WWM-05	Capricorn	Kgohloane	23°47'30.5"**S** 29°22'07.6"**E**	Lobed	Elliptic	Ochre brown
WWM-07	Capricorn	Moletjie-Moshate	23°36'55.5"**S** 29°16'13.5"**E**	Lobed	-	Fawn brown
WWM-08	Capricorn	Moletjie-Moshate	23°36'51.2"**S** 29°16'02.0"**E**	Lobed	-	Signal orange
WWM-09	Capricorn	Moletjie-Moshate	23°36'55.9"**S** 29°16'03.7"**E**	Lobed	-	Nut brown
WWM-14	Capricorn	Turfloop	23°53'18.7"**S** 29°44'53.8"**E**	Lobed	Round	Bright red orange
WWM-15	Capricorn	Turfloop	23°53'12.2"**S** 29°44'52.2"**E**	Lobed	Round	Tomato red
WWM-16	Waterberg	Bela-Bela	24°51'57.6"S 28°14'54.0"E	Lobed	Round	Ruby red
WWM-17(2)	Waterberg	Steiloop	23°25'56.3"**S** 28°37'33.1"**E**	Lobed	Round	Pastel yellow
WWM-21	Waterberg	Maeteletsa	23°26'28.6"**S** 28°10'52.1"**E**	Lobed	-	Jet black
WWM-23	Waterberg	Maeteletsa	23°26'28.6"**S** 28°10'52.1"**E**	Lobed	-	Luminous orange
WWM-24	Capricorn	Westernburg	23°54'27.2"**S** 29°25'23.1"**E**	Non-lobed	Elliptic	Olive brown
WWM-28	Sekhukhune	Nebo	24°44'13.3"**S** 30°33'39.0"**E**	Lobed	Round	Traffic red
WWM-33	Capricorn	Ga-Molepo	24°00'52.6"**S** 29°47'24.7"**E**	Lobed	Elliptic	Red orange
WWM-34	Capricorn	Ga-Molepo	24°01'11.1"**S** 29°47'05.0"**E**	Lobed	Round	Gaz’s beige
WWM-35(1)	Capricorn	Ga-Molepo	24°01'24.4"**S** 29°46'40.6"**E**	Lobed	-	Purple red
WWM-35(2)	Capricorn	Ga-Molepo	24°01'15.7"**S** 29°47'34.8"**E**	Lobed	-	Golden yellow
WWM-37(2)	Capricorn	Ga-Molepo	24°01'11.1"S 29°47'05.0"E	Lobed	-	Purple violet
WWM-38	Capricorn	Ga-Molepo	24°01'56.2"**S** 29°47'28.4"**E**	Lobed	Elliptic	Salmon pink
WWM-39	Capricorn	Ga-Mphela	23°43'19.2"**S** 29°12'01.4"**E**	Lobed	Broad-elliptic	Ruby red
WWM-40	Capricorn	Kgohloane	23°47'30.5"**S** 29°22'07.6"**E**	Lobed	Elliptic	Coral red
WWM-41(A)	Sekhukhune	Nebo	24°54'09.1"**S** 29°46'15.8"**E**	Lobed	Elliptic	Purple red
WWM-41(B)	Sekhukhune	Nebo	24°54'06.6"**S** 29°46'13.2"**E**	Lobed	Elliptic	Traffic orange
WWM-44	Sekhukhune	Nebo	24°54'02.0"**S** 29°46'01.8"**E**	Lobed	Round	Strawberry red
WWM-45(A)	Sekhukhune	Nebo	24°54'05.9"**S** 29°46'10.5"**E**	Lobed	-	Ruby red
WWM-45(B)	Sekhukhune	Nebo	24°54'07.2"S 29°46'13.2"E	Lobed	-	Pastel yellow
WWM-46	Sekhukhune	Nebo	24°54'07.2"S 29°46'13.2"E	Lobed	Elliptic	Signal red
WWM-47	Sekhukhune	Nebo	24°54'07.2"S 29°46'13.2"E	Lobed	Round	Ruby red
WWM-50	Sekhukhune	Nebo	24°54'02.2"S 29°46'13.9"E	Lobed	Elliptic	Traffic red
WWM-57	Sekhukhune	Nebo	24°54'02.2"S 29°46'13.9"E	Lobed	Round	Pearl orange
WWM-64	Capricorn	Ga-Mphela	23°39'46.0"**S** 29°19'16.4"**E**	Lobed	Elliptic	Golden yellow
WWM-64(2)	Capricorn	Ga-Mphela	23°39'46.0"**S** 29°19'16.4"**E**	Lobed	Elliptic	Pearl orange
WWM-65	Waterberg	Boltokwa	23°23'48.6"**S** 28°58'47.1"**E**	Lobed	Round	Purple red
WWM-66	Capricorn	Uitkyk 3	23°29'49.8"**S** 29°15'52.7"**E**	Lobed	Elliptic	Copper brown
WWM-67	Capricorn	Uitkyk 3	23°29'50.2"**S** 29°15'57.8"**E**	Lobed	Elliptic	Tomato red
WWM-68	Capricorn	Ga-Manamela	23°43'01.7"**S** 29°14'04.7"**E**	Lobed	Elliptic	Brown olive
WWM-76	Capricorn	Ga-Manamela	23°43'04.3"**S** 29°13'56.3"**E**	Lobed	Elliptic	Ochre yellow
WWM-79	Capricorn	Ga-Manamela	23°43'05.1"**S** 29°14'01.3"**E**	Lobed	Elliptic	Cream
WWM-81	Capricorn	Ga-Manamela	23°43'01.7"**S** 29°14'04.7"**E**	Lobed	Elliptic	Pastel yellow

4.2.2 Experimental design and crop establishment

Pot experiments were conducted under glasshouse condition at the Controlled Research Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37'37.5"**S** and 30°24'10.4"**E**). The first set of experiment to assess the drought tolerance of the genotypes was conducted from December 2020 to March 2021, and then repeated from March 2021 to May 2021. The study was designed as a 40 × 2 factorial experiment with 40 citron watermelon accessions grown under two water regimes namely: non-stressed (NS) and drought-stressed (DS). The experiment was laid in a completely randomized design (CRD) with three replications, giving 240 experimental units (8 L thermoform pots). Two seeds of each accession were planted per pot and later thinned to one plant per pot. Each accession was allocated to three pots resulting in three replications per accession for NS and DS conditions, respectively. Pots containing plants for each accession were placed randomly on raised benches in the glasshouse. Seeds were planted in a weed-free Gromor Potting Mix 30dm^3 growing media. Fertilizer was applied based on Gromor Potting Mix 30dm^3 properties. At planting, a basal fertilizer (N: P: K, 2:3:2) was applied at a rate of 50 g pot^{-1} (200 kg ha^{-1}). At 30 days after planting, a top-dressing fertilizer (Urea blended with single superphosphate in ratio 1:3) was applied at a rate of 50 g pot^{-1} (200 kg ha^{-1}).

Plants for each accession under DS and NS conditions for all replications were irrigated until the appearance of at least one or two male flower flowers corresponding to the development of six to ten fully expanded leaves. Thereafter, irrigation was withheld in the DS treatment for 14 days before taking measurements for gas exchange and chlorophyll fluorescence parameters. After measuring gas exchange and chlorophyll fluorescence parameters, followed by the resumption of irrigation for DS treatment maintaining the soil water potential between 80-85 cbar until maturity. Plants in NS condition were watered continuously to maintain soil water potential at approximately 15-20 cbar corresponding to field capacity until maturity. Irrometer moisture indicators (Irrometer Co., Riverside, California) were used to measure soil moisture based on tensiometric method (Kashyap and Kumar, 2021) (*see* Supplementary Figure 2). Informed irrigation decisions were done based on readings from DFM continuous logging soil moisture probes (*see* Supplementary Figure 3). The mean air temperature and relative humidity in the glasshouse were 25 ± 2 °C and 60 ± 3%, respectively.

4.2.3 Data collection

4.2.3.1 Gas exchange and chlorophyll fluorescence parameters

Gas exchange and chlorophyll fluorescence parameters were measured using LI-6400 XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) integrated with an infrared gas analyser (IRGA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm^2 leaf area, Licor Bioscience, Inc. Lincoln, Nebraska, USA). External leaf CO_2 concentration (C_a) and artificial saturating photosynthetic active radiation (PAR) were set at 400 µmol mol^{-1} and 1000 µmol m^{-2} s^{-1}. The temperature of the leaf was kept at 25 °C. Water flow rate and relative humidity were maintained at 500 µmol and 43%, respectively. The leaf-to-air vapour pressure deficit in the cuvette was maintained at 1.7 kPa to avoid stomatal closure due to low air humidity. Measurements were taken on the third half-fully formed leaf from the plant's tip between 08.30 and 11.30 a.m. by clamping the leaf inside the sensor head. Under both NS and DS conditions, measurements were taken from three plants for each accession.

The following gas exchange parameters were determined: stomatal conductance (gs), net CO_2 assimilation rate (A), transpiration rate (T), intercellular CO_2 concentration (C_i) and the ratio of intercellular and ambient CO_2 (C_i/C_a) concentrations. The ratio of net CO_2 assimilation rate to intercellular CO_2 concentration (A/C_i) was computed according to (Kitao et al., 2003). The ratio of A and gs was used to compute intrinsic water use efficiency (WUE_i) (Webster et al., 2016) and the ratio of A and T was used to calculate instantaneous water-use efficiency (WUE_{ins}) (Medrano et al., 2015).

To estimate chlorophyll fluorescence variables, a saturation flash intensity of 1300 mmol m^{-2} s^{-1} was applied. The following parameters were then recorded: the minimum (F_o') and maximum fluorescence (F_m') of light-adapted leaves under natural glasshouse conditions. The steady-state fluorescence (F_s) was also determined in light-adapted leaves under steady-state photosynthesis. Equation 4.1 was used to determine the variable fluorescence in light-adapted leaves, while Equation 4.2 calculated fluorescence changes.

$F_v' = F_m' - F_o'$ [Equation 4.1]

$\Delta F = F_m' - F_s$ [Equation 4.2] (Zlatev, 2014)

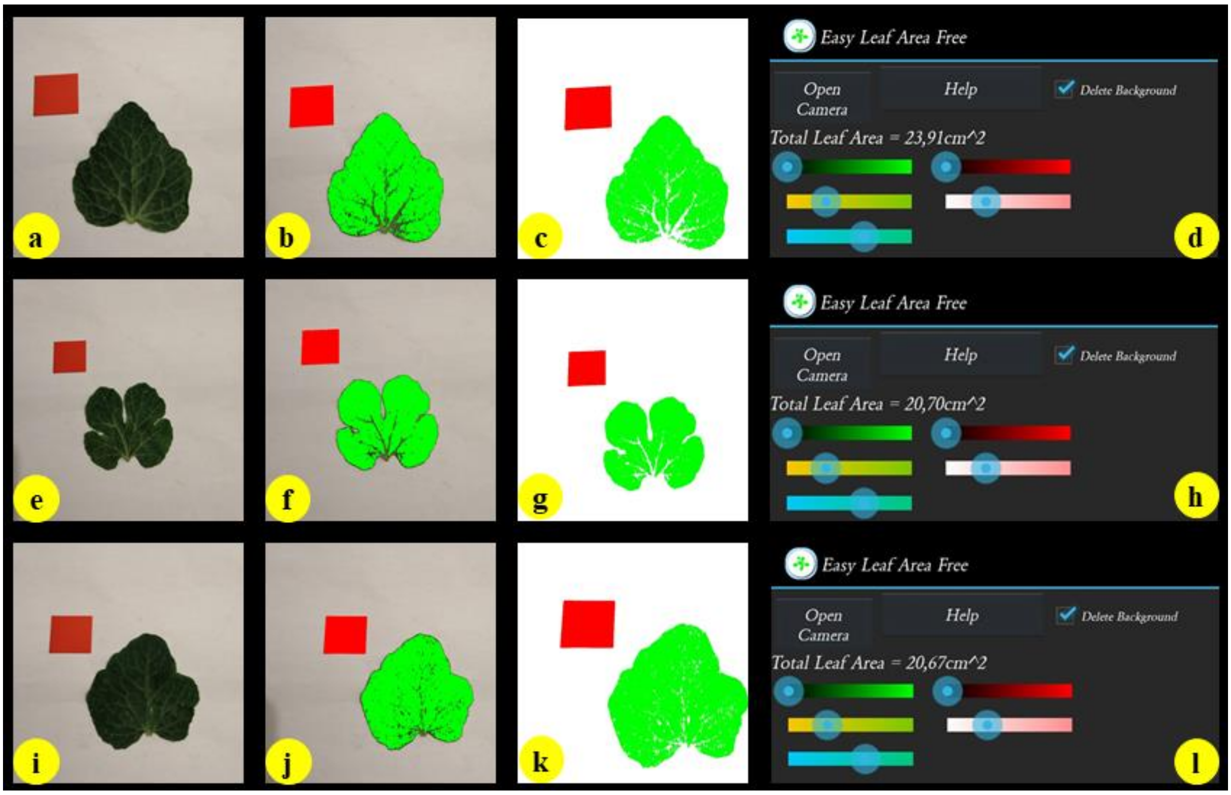
Additional chlorophyll fluorescence parameters were estimated according to Evans (2009): F_v'/F_m', the maximum quantum efficiency of photosystem II photochemistry, the effective quantum efficiency of photosystem II photochemistry (Φ_{PSII}), photochemical quenching (qP), non-photochemical quenching (qN), and electron transport rate (ETR). The ratio of ETR and A

was used to calculate a relative measure of electron transport to oxygen molecules. The alternative electron sink (*AES*) was calculated as the ratio of photosystem II effective quantum efficiency to CO_2 assimilation's quantum efficiency (*A*) (Ort and Baker, 2002). Gas exchange and chlorophyll fluorescence parameters were measured on fully expanded leaves.

4.2.4 Assessment of morphological traits

4.2.4.1 Specific leaf area

A fully expanded leaf at 42 days after planting was harvested per accession after recording gas exchange and chlorophyll fluorescence parameters and used to measure specific leaf area (SLA). Leaf area was measured using Easy Leaf Area software (Heaslon, University of California, California) according to Easlon and Bloom (2014). Easy Leaf Area uses a red calibration area of the known area in each image as a scale to calibrate leaf area estimates regardless of image source, eliminating the need to assess camera distance and focal length or measure ruler length manually. Total counts of green leaf pixels and red calibration pixels were used to estimate leaf area, according to leaf area = (green pixel count) × (calibration area/red pixel count) (Figure 4.1).



Total Leaf Area = 23,91cm^2

Total Leaf Area = 20,70cm^2

Total Leaf Area = 20,67cm^2

Figure 4.1: Raw and processed photographs of citron watermelon leaves. Unprocessed images (**a**, **e** and **i**), images after greenest and reddest pixel selection (**b**, **f** and **j**), and images after final automated processing (**c**, **g** and **k**) with the delete background option selected (**d**, **h** and **l**)

Leaves were oven-dried at 75 °C for 72 h until the weight was constant. Leaf dry weight (LDW) was measured using an electronic balance (Adam AAA 100L, Adam Equipment, South Africa). The SLA was calculated as the ratio of leaf area (cm^2) to leaf dry weight (g) according to Qin et al. (2019) using Equation 4.3.

$$\text{SLA} = \frac{\text{Leaf Area }(\text{cm}^2)}{\text{Leaf Dry Weight }(\text{g})}$$ [Equation 4.3] (Qin et al., 2019)

4.2.4.2 *Root morphological and architectural phenes*

The shovelomics approach was used to quantify root phenes (Arifuzzaman et al., 2019; Bucksch et al., 2014). The shovelomics procedure captures most of the root system biomass within the excavation area. After excavation, the shoot was cut from the root using secateurs at 5 cm above the soil level and washed in 5% sodium hypochlorite to remove soil and microbiome on the roots. Washed roots were blotted with a paper towel and pressed onto newsprint for 7 days. Pressed dry roots were scanned using a Konica Minolta Bizhub C224 Multifunction Printer. Root system architectural (RSA) traits from excavated root images were quantified using a user-assisted root image analysis package (RootSnap Version 1.3.2.25, CID Bio-Science Inc.). The following RSA traits were measured: taproot length (TRL), root dry mass (RDM) and estimated rooting percentage (ERP).

4.2.4.3 *Yield and drought tolerance indices*

Fresh citron watermelon fruits were harvested 90 days after planting and weight was determined using a precision scale (UW4200H Shimadzu, Japan) for all treatments. Various drought tolerance indices [stress susceptibility index (SSI), tolerance (TOL), mean productivity index (MPI), stress tolerance index (STI), geometric mean productivity (GMP), yield index (YI) and yield stability index (YSI)] were calculated based on fruit yield under NS and DS conditions using equations presented in Table 4.2.

Table 4.2: Drought tolerance indices used to evaluate citron watermelon accessions

Drought tolerance indices	**Equation**	**Reference**
Stress Susceptibility Index (SSI)	$\mathbf{SSI} = \frac{\left[1 - \frac{Ys}{Yp}\right]}{\left[1 - \frac{\bar{\bar{\bar{Y}}}s}{\bar{\bar{\bar{Y}}}p}\right]}$	(Fischer and Maurer, 1978)[Equation 4.4]
Tolerance (TOL)	$\mathbf{TOL} = Y_P - Y_s$	(Schneider et al., 1997) [Equation 4.5]
Mean Productivity Index (MPI)	$\mathbf{MPI} = \frac{Y_P + Y_s}{2}$	(Rosielle and Hamblin, 1981) [Equation 4.6]
Stress Tolerance Index (STI)	$\mathbf{STI} = \frac{Y_P \times Y_s}{\left(\bar{Y}_p\right)^2}$	(Fernandez, 1992) [Equation 4.7]
Geometric Mean Productivity (GMP)	$\mathbf{GMP} = \sqrt{(Y_P)(Y_s)}$	(Fernandez, 1992) [Equation 4.8]

Yield Index (YI)	$\mathbf{YI} = \frac{Ys}{\bar{Y}s}$	(Gavuzzi et al., 1997) [Equation 4.9]
Yield Stability Index	$\mathbf{YSI} = \frac{Y_s}{Y_P}$	(Boyer, 1982) [Equation 4.10]

*$\boldsymbol{Yp}$; yield of each genotype under non-stress, $\boldsymbol{Ys}$; yield of each genotype under stress, $\bar{\boldsymbol{Y}}\boldsymbol{s}$; mean yield of all genotypes in non-stress conditions, $\bar{\boldsymbol{Y}}\boldsymbol{p}$; mean yield of all genotypes in stress conditions

4.2.5 Statistical analysis

Averaged data across two seasons was subjected to analysis of variance using Genstat 20th edition (VSN International, Hempstead, United Kingdom). Means were separated using Fisher's protected least significant difference (LSD) test when treatments showed significant differences on measured parameters at a 5% level of significance. Agglomerative Hierarchical Clustering (AHC) was performed using drought tolerance indices according to Ward's method using squared Euclidean distance to measure similarity using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France 2017) Principal component analysis (PCA) and the biplot diagrams were exploited to identify tolerant and susceptible genotypes using XLSTAT. Pearson correlation heatmaps were drawn based on mean values using GraphPad Prism Version 9.2.0 (GraphPad Software, Inc.) and Origin Pro 2021b (OriginLab Corporation).

4.3 Results

4.3.1 Leaf gas exchange and chlorophyll fluorescence parameters

ANOVA for evaluated gas exchange and chlorophyll fluorescence parameters indicated that the effects of irrigation regime, genotype, and interaction were significantly different for most traits (Table 4.3). Drought stress significantly decreased stomatal conductance (*gs*), net CO_2 assimilation rate (*A*), transpiration rate (*T*) and CO_2 assimilation rate/intercellular CO_2 concentration (A/C_i) among the evaluated accessions (Figure 4.2a, b, c and e). Accessions WWM-65, WWM-81, WWM-44 and WWM-28 recorded *gs* values of ≥ 0.137 mmol m^{-2} s^{-1} under NS condition, whereas accessions WWM-33, WWM-35(2), WWM-09 and WWM-35(1) recorded *gs* values of ≤ 0.019 mmol m^{-2} s^{-1} under DS condition. For *T*, accessions WWM-07, WWM-03, WWM-45(A) and WWM-41(B) recorded values ≥ 9.494 mmol H_2O m^{-2} s^{-1} under NS condition, compared to—accessions WWM-33, WWM-65 and WWM-15 which recorded *T* values ≤ 2.075 mmol H_2O m^{-2} s^{-1} under DS condition. Under NS condition, *A* values ≥ 41.490 µmol CO_2 m^{-2} s^{-1} were recorded for WWM-44, WWM-28, WWM-46 and WWM-45(A), whereas *A* values ≤ 14.31 µmol CO_2 m^{-2} s^{-1} was recorded for accessionsWWM-68, WWM-44 and WWM-57 under DS condition.

Drought stress increased both intrinsic water-use efficiency (WUE_i) and instantaneous water-use efficiency (WUE_{inst}) (Figure 4.2g and h). Highest WUE_i (1225.600 µmol (CO_2) m^{-2}) was recorded under DS condition for accession WWM-35(2) (Table 4.5). Accession WWM-15 recorded the highest WUE_{inst} (9.540 µmol. mol^{-1}) under DS condition compared with other accessions under NS condition (Table 4.4).

Table 4.3 highlights the effect of water stress on chlorophyll fluorescence parameters among the citron watermelon accessions. Chlorophyll fluorescence parameters showed a significant genotype × water regime interaction, indicating that the evaluated accessions responded differently under NS and DS conditions. Genotypic variability with respect to (maximum fluorescence) F_m' was observed under both conditions. The mean value for the $F_{v'}/F_{m'}$ of all evaluated accessions was higher under DS condition (0.191) than NS condition (0.044) (Figure 2k).

The effective quantum efficiency of PSII photochemistry (Φ_{PSII}) varied significantly among the tested accessions under both NS and DS conditions (Table 4.5). Values for Φ_{PSII} ranged from 0.127 (WWM-35(1)) to 0.332 (WWM-15) under NS condition, and from 0.052 (WWM-50) to 0.260 (WWM-38) under DS condition. The mean Φ_{PSII} values in all accessions was higher under DS condition (0.213) than NS condition (0.148) (Figure 4.2l). The following accessions namely WWM-41(A), WWM-02, WWM-03 and WWM-05) recorded Φ_{PSII} values $\geq$ 0.270 under NS condition, whereas accessions WWM-45(A), WWM-57, WWM-23 and WWM-38 recorded Φ_{PSII} values of $\geq$ 0.226 under DS condition.

There were varying genotypic responses for non-photochemical quenching (qN) under NS and DS conditions. The mean for qN was significantly higher under DS conditions (3.107) compared to NS conditions (1.603) (Figure 4.2n). The qN ranged from 2.334 to 1.394 under NS condition (Table 4.4), and from 2.120 to 3.692 under DS condition (Table 4.5). Accessions (WWM-37(2), WWM-45(B), WWM-64 and WWM-34) recorded qN values $\geq$ 2.075 under NS condition, whereas accessions WWM-64, WWM-15, WWM-28 and WWM-46 recorded qN values of $\geq$ 3.569 under DS condition.

Table 4.3: Analysis of variance showing mean squares and significant tests for leaf gas exchange and chlorophyll fluorescence parameters of 40 citron watermelon landrace accessions evaluated under drought-stressed and non-stressed conditions

		Leaf gas exchange parameters							
Source of variation	**d.f**	***gs***	***T***	***A***	**C_i**	**A/C_i**	**C_i/C_a**	**WUE_i**	**WUE_{inst}**
Genotype	39	3.259×10^{-4ns}	2.394^{*}	13.71^{ns}	4030^{**}	3.423×10^{-4}	0.025^{**}	44220^{*}	2.548^{*}
Irrigation	1	0.529^{**}	1517.994^{**}	27975.43^{**}	$5.284\times10^{-5**}$	0.343^{**}	3.302^{**}	$8.637\times10^{-6**}$	67.828^{**}
Genotype×Irrigation	39	3.649×10^{-4}	2.248^{ns}	27.01^{ns}	2328^{**}	$4.363\times10^{-4*}$	0.015^{**}	55156^{ns}	3.227^{*}
Residual	160	2.845×10^{-4}	1.583	20.26	1110	2.759×10^{-4}	6.936×10^{-3}	59407	2.223

		Chlorophyll fluorescence parameters								
Source of variation	**d.f**	**F_o'**	**F_m'**	**F_v/F_m**	**Φ_{PSII}**	***qP***	***qN***	***ETR***	***ETR/A***	***AES***
Genotype	39	2731^{*}	141124^{**}	0.027^{**}	0.006^{**}	0.012^{**}	0.323^{**}	$1.938\times10^{9**}$	$5.680\times10^{6**}$	9023^{*}
Irrigation	1	988778^{**}	$3462\times10^{7**}$	3.692^{**}	0.417^{**}	1.629^{**}	106.437^{**}	$1.473\times10^{11**}$	$8.059\times10^{7**}$	$8.158\times10^{6**}$
Genotype×Irrigation	39	2109^{*}	96136^{*}	0.027^{**}	$6.679\times10^{-3**}$	0.012^{**}	0.266^{**}	$1.947\times10^{9**}$	$6.793\times10^{6**}$	10473
Residual	160	1391	49063	0.011	2.722×10^{-3}	0.005	0.123	7.533×10^{8}	2.732×10^{6}	6072

d.**f**; degrees of freedom, ***gs***; stomatal conductance, ***T***; transpiration rate, ***A***; net CO_2 assimilation rate, **A/C_i**; CO_2 assimilation rate/intercellular CO_2 concentration, ***Ci***; intercellular CO_2 concentration, **C_i/C_a**, ratio of intercellular and atmospheric CO_2, **WUE_i**; intrinsic water use efficiency, **WUE_{ins}**; instantaneous water-use efficiency, **F_v/F_m**; maximum quantum efficiency of photosystem II photochemistry, **Φ_{PSII}**; the effective quantum efficiency of PSII photochemistry, ***qP***; photochemical quenching, ***qN***; non-photochemical quenching, ***ETR***; electron transport rate, ***ETR/A***; relative measure of electron transport to oxygen molecules, ***AES***; alternative electron sinks . * and ** denote significant at 5 and 1% probability levels, respectively. ns, non-significant

Significant genotypic variation for *ETR* were observed under NS (Table 4.4) and DS conditions (Table 4.5). Under NS condition, accessions WWM-03, WWM-15, WWM-41(A), WWM-34 and WWM-68 recorded the highest *ETR* (≥ 140379 µmol e^{-1} m^{-2} s^{-1}), whereas WWM-35(1), WWM-40, WWM-38, WWM-41(B) and WWM-45(A) recorded the lowest *ETR* (≤ 93652 µmol e^{-1} m^{-2} s^{-1}). Under DS condition, accessions WWM-38, WWM-57, WWM-45(A) and WWM-14 recorded the highest *ETR* (≥ 107335 µmol e^{-1} m^{-2} s^{-1}), whereas WWM-09, WWM-28, WWM-81 and WWM-41(B) recorded the lowest *ETR* value (≤ 43637 µmol e^{-1} m^{-2} s^{-1}). DS significantly reduced *ETR* of all accessions. Under NS, the average *ETR* value was 119000.875 µmol e^{-1} m^{-2} s^{-1}, higher than 64007.325 µmol e^{-1} m^{-2} s^{-1} recorded under DS condition.

Drought stress significantly increased the relative measure of electron transport to oxygen molecules (*ETR/A*) (Figure 4.2p). Highest *ETR/A* (≥ 8148 µmol e µmol^{-1} CO_2) was recorded under DS condition in accessions WWM-57, WWM-38, WWM-45(A) and WWM-65. Also, DS significantly increased *AES* compared to NS condition. Under DS condition, *AES* ranged from 284.300 to 618.100 and under NS condition, and from 57.340 to 287.200 under DS condition (Tables 4.4 and 4.5).

Table 4.4: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon accessions under non-stressed condition

	Leaf gas exchange parameters								**Chlorophyll fluorescence parameters**								
Accession	***gs***	***T***	***A***	C_i	A/C_i	Ci/C_a	WUE_i	WUE_{inst}	F_o'	F_m'	F_v/F_m	Φ_{PSII}	***qP***	***qN***	***ETR***	***ETR/A***	***AES***
WWM-02	**0.093^{c}**	8.186^{a}	**30.090^{a}**	279.300^{bc}	0.110^{a}	0.698^{bc}	321.100^{a}	3.836^{ab}	301.300^{a}	454.100^{ab}	0.337^{b}	0.274^{abc}	0.524^{ab}	1.507^{a}	151067^{abc}	5156^{ab}	73.240^{b}
WWM-03	0.117^{abc}	9.667^{a}	31.830^{a}	332.400^{abc}	0.097^{a}	0.831^{abc}	271.600^{a}	**3.309^{b}**	343.700^{a}	450^{ab}	0.693^{ab}	0.296^{ab}	**0.589^{a}**	1.635^{a}	**190996^{a}**	**6013^{a}**	**57.340^{b}**
WWM-05	0.109^{bc}	6.987^{a}	30.970^{a}	279.600^{bc}	0.110^{a}	0.699^{bc}	284.700^{a}	4.469^{ab}	306.900^{a}	524.400^{ab}	0.411^{ab}	0.193^{abc}	0.400^{ab}	1.711^{a}	106490^{abcd}	3708^{abc}	60.740^{b}
WWM-07	0.117^{abc}	9.494^{a}	32.350^{a}	335.200^{abc}	0.096^{a}	0.838^{abc}	279^{a}	3.429^{ab}	344.900^{a}	639.600^{ab}	0.462^{ab}	0.200^{abc}	0.467^{ab}	1.863^{a}	108153^{abcd}	3501^{abc}	73.440^{b}
WWM-08	0.136^{abc}	7.263^{a}	33.130^{a}	322^{abc}	0.103^{a}	0.805^{abc}	**245.700^{a}**	4.658^{ab}	364.500^{a}	513.300^{ab}	0.379^{ab}	0.222^{abc}	0.479^{ab}	1.622^{a}	122453^{abcd}	3855^{abc}	70.590^{b}
WWM-09	0.133^{abc}	7.656^{a}	37.760^{a}	368.100^{abc}	0.104^{a}	0.920^{abc}	288^{a}	4.990^{ab}	324.300^{a}	517.900^{ab}	0.368^{ab}	0.189^{abc}	0.444^{ab}	1.594^{a}	104235^{abcd}	2808^{abc}	72.650^{b}
WWM-14	0.107^{bc}	7.684^{a}	35.100^{a}	347.100^{abc}	0.101^{a}	0.868^{abc}	327.300^{a}	4.657^{ab}	329.100^{a}	569.200^{ab}	0.395^{ab}	0.236^{abc}	0.463^{ab}	1.630^{a}	129892^{abcd}	3814^{abc}	72.130^{b}
WWM-15	0.111^{bc}	**6.008^{a}**	35.180^{a}	288.100^{abc}	0.125^{a}	0.720^{abc}	317.200^{a}	5.877^{ab}	315.600^{a}	652.200^{ab}	0.426^{ab}	**0.332^{a}**	0.490^{ab}	1.750^{a}	180027^{ab}	5259^{ab}	84.080^{b}
WWM-16	0.128^{abc}	7.729^{a}	35.270^{a}	398.400^{abc}	**0.088^{a}**	0.996^{abc}	277.100^{a}	4.606^{ab}	295.700^{a}	449.400^{ab}	0.335^{b}	0.183^{abc}	**0.261^{b}**	1.514^{a}	101212^{bcd}	3019^{abc}	71.770^{b}
WWM-17(2)	0.115^{bc}	8.733^{a}	37.180^{a}	386^{abc}	0.097^{a}	0.965^{abc}	324.100^{a}	4.427^{ab}	306.200^{a}	456.900^{ab}	0.318^{b}	0.218^{abc}	0.375^{ab}	1.485^{a}	120236^{abcd}	3271^{abc}	73.210^{b}
WWM-21	0.137^{abc}	6.915^{a}	35.890^{a}	345.200^{abc}	0.105^{a}	0.863^{abc}	265^{a}	5.204^{ab}	319.800^{a}	494.100^{ab}	0.352^{ab}	0.197^{abc}	0.302^{b}	1.546^{a}	108436^{abcd}	3154^{abc}	71.610^{b}
WWM-23	0.131^{abc}	8.604^{a}	36.110^{a}	302.200^{abc}	0.123^{a}	0.756^{abc}	275.100^{a}	4.204^{ab}	331^{a}	668.700^{ab}	0.428^{ab}	0.247^{abc}	0.488^{ab}	1.956^{a}	136544^{abc}	3919^{abc}	66.390^{b}
WWM-24	0.129^{abc}	6.401^{a}	37.630^{a}	363.900^{abc}	0.103^{a}	0.910^{abc}	294.800^{a}	5.892^{ab}	314.500^{a}	467.400^{ab}	0.327^{b}	0.254^{abc}	0.472^{ab}	1.486^{a}	140167^{abc}	3738^{abc}	62.320^{b}
WWM-28	**0.167^{a}**	9.429^{a}	41.630^{a}	408.700^{ab}	0.102^{a}	1.022^{ab}	265.500^{a}	4.486^{ab}	296.500^{a}	417.200^{ab}	0.287^{b}	0.216^{abc}	0.288^{b}	1.405^{a}	119198^{abcd}	2883^{abc}	61.230^{b}
WWM-33	0.119^{abc}	8.846^{a}	37.500^{a}	358.100^{abc}	0.106^{a}	0.895^{abc}	313.900^{a}	4.420^{ab}	308.200^{a}	475.700^{ab}	0.352^{b}	0.199^{abc}	0.288^{b}	1.543^{a}	110054^{abcd}	3006^{abc}	73.750^{b}
WWM-34	0.131^{abc}	8.228^{a}	36.940^{a}	407.700^{ab}	0.090^{a}	1.019^{ab}	280.600^{a}	4.474^{ab}	373.100^{a}	690.700^{ab}	**0.791^{a}**	0.260^{abc}	0.365^{ab}	**2.334^{a}**	143560^{abc}	4251^{abc}	**287.200^{a}**
WWM-35(1)	0.125^{abc}	7.954^{a}	38.970^{a}	375.600^{abc}	0.107^{a}	0.939^{abc}	310.400^{a}	5^{ab}	301.400^{a}	464.600^{ab}	0.314^{b}	**0.127^{c}**	0.404^{ab}	1.512^{a}	**42865^{d}**	**1107^{c}**	78.250^{b}
WWM-35(2)	0.120^{abc}	8.289^{a}	38.170^{a}	341.100^{abc}	0.115^{a}	0.853^{abc}	330.300^{a}	4.687^{ab}	316.100^{a}	482.900^{ab}	0.344^{b}	0.210^{abc}	0.389^{ab}	1.524^{a}	116214^{abcd}	3087^{abc}	78.290^{b}
WWM-37(2)	0.124^{abc}	6.812^{a}	38.160^{a}	323.100^{abc}	0.118^{a}	0.808^{abc}	310^{a}	5.583^{ab}	329.100^{a}	669.600^{ab}	0.399^{ab}	0.223^{abc}	0.334^{ab}	2.075^{a}	123308^{abcd}	3336^{abc}	70.430^{b}
WWM-38	0.129^{abc}	6.722^{a}	40.670^{a}	378.500^{abc}	0.108^{a}	0.946^{abc}	325.400^{a}	6.053^{ab}	319.400	845.800^{ab}	0.497^{ab}	0.145^{bc}	0.475^{ab}	1.913^{a}	80097^{cd}	1994^{bc}	67.520^{b}
WWM-39	0.118^{abc}	7.877^{a}	38.690^{a}	321.800^{abc}	0.122^{a}	0.804^{abc}	326.600^{a}	4.983^{ab}	344.200^{a}	526.600^{ab}	0.409^{ab}	0.214^{abc}	0.457^{ab}	1.717^{a}	118147^{abcd}	3221^{abc}	76.360^{b}
WWM-40	0.112^{bc}	7.089^{a}	40.670^{a}	**266.600^{c}**	**0.157^{a}**	**0.666^{c}**	**362.800^{a}**	5.757^{ab}	304.800^{a}	427.100^{ab}	**0.277^{b}**	0.133^{c}	0.353^{ab}	1.735^{a}	73568^{cd}	1842^{bc}	82.130^{b}
WWM-41(A)	0.122^{abc}	8.956^{a}	41.460^{a}	325.300^{abc}	0.128^{a}	0.813^{abc}	343.500^{a}	4.644^{ab}	330.900^{a}	603.600^{ab}	0.392^{ab}	0.273^{abc}	0.488^{ab}	1.754^{a}	150892^{abc}	3650^{abc}	74.220^{b}
WWM-41(B)	0.118^{abc}	**10.805^{a}**	39.320^{a}	363.700^{abc}	0.109^{a}	0.909^{abc}	334.100^{a}	3.658^{ab}	306.900^{a}	460.400^{ab}	0.328^{b}	0.163^{bc}	0.365^{ab}	1.492^{a}	90166^{cd}	2394^{bc}	60.020^{b}
WWM-44	0.150^{ab}	6.750^{a}	41.490^{a}	297.700^{abc}	0.139^{a}	0.744^{abc}	278.100^{a}	**6.209^{a}**	332.300^{a}	524.900^{ab}	0.367^{ab}	0.231^{abc}	0.375^{ab}	1.581^{a}	127906^{abcd}	3082^{abc}	66.300^{b}
WWM-45(A)	0.129^{abc}	10.380^{a}	**43.310^{a}**	303^{abc}	0.153^{a}	0.758^{abc}	343.500^{a}	4.453^{ab}	329.300^{a}	590.400^{ab}	0.415^{ab}	0.169^{bc}	0.524^{ab}	1.766^{a}	93652^{bcd}	2168^{bc}	75.810^{b}
WWM-45(B)	0.115^{bc}	7.088^{a}	40.890^{a}	320^{abc}	0.128^{a}	0.800^{abc}	356.600^{a}	5.766^{ab}	324.700^{a}	493.400^{ab}	0.652^{ab}	0.214^{abc}	0.333^{ab}	2.140^{a}	118381^{abcd}	2906^{abc}	66.230^{b}
WWM-46	0.117^{abc}	7.981^{a}	42.200^{a}	306.800^{abc}	0.138^{a}	0.767^{abc}	360.700^{a}	5.364^{ab}	359.800^{a}	656.500^{ab}	0.400^{ab}	0.214^{abc}	0.441^{ab}	1.753^{a}	118436^{abcd}	2789^{abc}	90.090^{b}
WWM-47	0.123^{abc}	9.216^{a}	40.890^{a}	379.600^{abc}	0.108^{a}	0.949^{abc}	331.800^{a}	4.596^{ab}	344.300^{a}	631.100^{ab}	0.376^{ab}	0.177^{abc}	0.355^{ab}	1.727^{a}	117078^{abcd}	2907^{abc}	73.060^{b}
WWM-50	0.119^{abc}	8.649^{a}	41.020^{a}	348.400^{abc}	0.118^{a}	0.871^{abc}	343.100^{a}	5.058^{ab}	337.900^{a}	554^{ab}	0.343^{b}	0.187^{abc}	0.307^{b}	1.594^{a}	103210^{bcd}	2515^{bc}	72.400^{b}
WWM-57	0.129^{abc}	7.193^{a}	40.890^{a}	**415.600^{a}**	0.100^{a}	**1.039^{a}**	315.900^{a}	5.659^{ab}	299.800^{a}	**855.500^{a}**	0.499^{ab}	0.238^{abc}	0.501^{ab}	1.759^{a}	131488^{abc}	3346^{abc}	77.130^{b}
WWM-64	0.113^{bc}	7.961^{a}	39.920^{a}	414.700^{a}	0.096^{a}	1.037^{a}	352.200^{a}	5.050^{ab}	**376.200^{a}**	850.900^{ab}	0.485^{ab}	0.237^{abc}	0.385^{ab}	2.171^{a}	128502^{abcd}	3262^{abc}	76.620^{b}
WWM-64(2)	0.130^{abc}	7.044^{a}	41.070^{a}	352.500^{abc}	0.122^{a}	0.881^{abc}	319.500^{a}	5.814^{ab}	341.900^{a}	607.300^{ab}	0.400^{ab}	0.204^{abc}	0.363^{ab}	1.736^{a}	111072^{abcd}	2765^{abc}	81.220^{b}
WWM-65	0.137^{abc}	8.652^{a}	40.740^{a}	337.400^{abc}	0.121^{a}	0.844^{abc}	295.800^{a}	4.882^{ab}	342.400^{a}	618.300^{ab}	0.399^{ab}	0.238^{abc}	0.415^{ab}	1.760^{a}	100203^{bcd}	2476^{bc}	59.810^{b}
WWM-66	0.125^{abc}	6.936^{a}	41.120^{a}	382^{abc}	0.107^{a}	0.955^{abc}	337.900^{a}	5.912^{ab}	**290.800^{a}**	405.400^{ab}	0.282^{b}	0.172^{abc}	0.462^{ab}	**1.394^{a}**	95123^{bcd}	2399^{bc}	58.630^{b}
WWM-67	0.122^{abc}	9.028^{a}	34.740^{a}	306^{abc}	0.120^{a}	0.765^{abc}	284.700^{a}	4.024^{ab}	325.300^{a}	491.200^{ab}	0.336^{b}	0.205^{abc}	0.408^{ab}	1.512^{a}	113567^{abcd}	3324^{abc}	61.770^{b}
WWM-68	0.125^{abc}	7.281^{a}	36.210^{a}	323.400^{abc}	0.113^{a}	0.809^{abc}	288.800^{a}	5.034^{ab}	366.100^{a}	**235.200^{ab}**	0.591^{ab}	0.254^{abc}	0.514^{ab}	1.657^{a}	140379^{abc}	3963^{abc}	58.750^{b}
WWM-76	0.119^{abc}	7.108^{a}	36.490^{a}	361.200^{abc}	0.101^{a}	0.903^{abc}	316.700^{a}	5.164^{ab}	328.300^{a}	507.400^{ab}	0.349^{b}	0.250^{abc}	0.368^{ab}	1.541^{a}	137961^{abc}	3800^{abc}	78.900^{b}
WWM-79	0.116^{abc}	8.096^{a}	36.160^{a}	288.900^{abc}	0.126	0.722^{abc}	306.800^{a}	4.616^{ab}	316.600^{a}	477.400^{ab}	0.337	0.187^{abc}	0.380^{ab}	1.508^{a}	103675^{bcd}	2863^{abc}	68.760^{b}

WWM-81	0.137^{abc}	8.206^{a}	35.610^{a}	312^{abc}	0.115^{a}	0.780^{abc}	260.400^{a}	4.390^{ab}	322.300^{a}	462.900^{ab}	0.304^{b}	0.200^{abc}	0.343^{ab}	1.436^{a}	110712^{abcd}	3203^{abc}	84.020^{b}
LSD	0.025	2.505	8.712	66.250	0.036	0.166	83.280	1.383	60.370	305.300	0.216	0.079	0.134	0.505	42911.900	1690	96.380
CV (%)	12.400	19.300	14.200	11.900	19.400	11.900	16.600	17.400	11.400	34.300	32.900	22.800	20.000	18.400	22.400	32.100	77.300
P-value	0.007	0.062	0.298	<.001	0.065	<.001	0.408	0.002	0.450	0.128	0.003	0.001	<.001	0.077	<.001	<.001	0.422

Different upper-case letters within a column indicate significant difference among genotypes. ***gs***; (mmol m^{-2} s^{-1}), ***T***; (mmol H_2O m^{-2} s^{-1}), ***A***; (µmol CO_2 m^{-2} s^{-1}), ***A/Ci***; (µmol. mol m^{-1}), ***Ci***; (µmol. mol m^{-1}), ***WUEi***; [(µmol (CO_2) m^{-2}]; ***WUEins***, (µmol. mol^{-1}), ***Fv/Fm***; (ratio); ***ΦPSII***, the effective quantum efficiency of PSII photochemistry; ***qP***, photochemical quenching; ***qN***, non-photochemical quenching; ***ETR***, (µmol e^{-1} m^{-2} s^{-1}); ***ETR/A***, (µmol e $µmol^{-1}$ CO_2); ***AES***, alternative electron sinks

Table 4.5: Means of leaf gas exchange and chlorophyll fluorescence parameters of citron watermelon accessions under drought-stressed condition

	Leaf gas exchange parameters								**Chlorophyll fluorescence parameters**								
Genotype	***gs***	***T***	***A***	C_i	A/C_i	Ci/C_a	WUE_i	WUE_{inst}	F_o'	F_m'	F_v/F_m	Φ_{PSII}	***qP***	***qN***	***ETR***	***ETR/A***	***AES***
WWM-02	0.023^{a}	2.453^{a}	16.420^{a}	446ab	0.037	1.115^{ab}	728^{a}	7.381^{a}	**387^{b}**	825^{cd}	0.527	0.074^{bc}	0.219^{ab}	**2.120^{b}**	64597^{ab}	4055^{ab}	**284.300^{b}**
WWM-03	0.025^{a}	2.694^{a}	16.930^{a}	439.500^{ab}	0.039^{a}	1.099^{ab}	751.800^{a}	8.023^{a}	420.800^{b}	1105^{abcd}	0.587^{cd}	0.115^{abc}	0.202^{ab}	2.539^{ab}	58170^{ab}	3515^{ab}	396.400^{ab}
WWM-05	0.029^{a}	2.763^{a}	16.780^{a}	393.600^{b}	0.043^{a}	0.984^{b}	674.500^{a}	6.874^{a}	458.700^{ab}	1331^{abcd}	0.637^{bcd}	0.140^{abc}	0.259^{ab}	2.838^{ab}	75044^{ab}	4503^{ab}	439.100^{ab}
WWM-07	0.022^{a}	3.789^{a}	17.360^{a}	400.800^{ab}	0.044^{a}	1.002^{ab}	864^{a}	5.338^{a}	479.400^{ab}	1338^{abcd}	0.641^{bcd}	0.089^{abc}	0.179^{ab}	2.793^{ab}	48295^{ab}	2787^{ab}	400.200^{ab}
WWM-08	0.020^{a}	3.570^{a}	16.970^{a}	423.900^{ab}	0.040^{a}	1.060^{ab}	877^{a}	4.739^{a}	480.200^{ab}	1487^{abcd}	0.677^{abcd}	0.098^{abc}	0.260^{ab}	3.474^{a}	52490^{ab}	3178^{ab}	613^{a}
WWM-09	0.017^{a}	2.677^{a}	15.070^{a}	438.500^{ab}	0.035^{a}	1.096^{ab}	878.400^{a}	5.672^{a}	452.900^{ab}	1259^{abcd}	0.629^{bcd}	0.082^{abc}	0.220^{ab}	2.719^{ab}	43637^{b}	2898^{ab}	**618.100^{a}**
WWM-14	0.033^{a}	2.811^{a}	17.220^{a}	445^{ab}	0.038^{a}	1.113^{ab}	629.200^{a}	6.313^{a}	490.800^{ab}	1548^{abcd}	0.824^{ab}	0.201^{abc}	0.275^{ab}	2.437^{ab}	107335^{ab}	5908^{ab}	502.100^{ab}
WWM-15	0.034^{a}	**1.531^{a}**	14.690^{a}	405.200^{ab}	0.036^{a}	1.013^{ab}	497.900^{a}	**9.540^{a}**	422.600^{b}	1027^{abcd}	0.664^{abcd}	0.065^{bc}	0.176^{ab}	3.619^{a}	65194^{ab}	5242^{ab}	388.100^{ab}
WWM-16	0.022^{a}	2.608^{a}	15.950^{a}	416.300^{ab}	0.038^{a}	1.041^{ab}	787.100^{a}	6.350^{a}	428.300^{ab}	1444^{abcd}	0.703^{abcd}	0.095^{abc}	0.268^{ab}	3.371^{ab}	50587^{ab}	3100^{ab}	407.900^{ab}
WWM-17(2)	0.033^{a}	3.363^{a}	17.680^{a}	438.200^{ab}	0.041^{a}	1.096^{ab}	614.500^{a}	5.560^{a}	491.100^{ab}	1422^{abcd}	0.655^{bcd}	0.114^{abc}	0.286^{ab}	2.907^{ab}	71007^{ab}	3992^{ab}	446^{ab}
WWM-21	0.023^{a}	3.194^{a}	19^{a}	393.600b	**0.049^{a}**	0.984^{b}	979.200^{a}	6.048^{a}	444.900^{ab}	1343^{abcd}	0.667^{abcd}	0.107^{abc}	0.239^{ab}	3.010^{ab}	46465^{ab}	2388^{ab}	400.200^{ab}
WWM-23	0.038^{a}	2.598^{a}	16.500^{a}	446.600^{ab}	0.037^{a}	1.117^{ab}	689.400^{a}	6.306^{a}	421.200^{b}	1045^{abcd}	0.605^{cd}	0.238^{ab}	**0.324^{a}**	2.456^{ab}	51786^{ab}	3197^{ab}	452.200^{ab}
WWM-24	0.031^{a}	3.474^{a}	17.990^{a}	461.500^{ab}	0.039^{a}	1.154^{ab}	671.300^{a}	5.471^{a}	470.600^{ab}	1450^{abcd}	0.669^{abcd}	0.076^{bc}	0.202^{ab}	3.082^{ab}	55051^{ab}	3272^{ab}	459.400^{ab}
WWM-28	0.026^{a}	2.727^{a}	17.750^{a}	470.700^{ab}	0.038^{a}	1.177^{ab}	677.400^{a}	7.291^{a}	467.100^{ab}	**1761^{a}**	**0.868^{a}**	0.081^{abc}	0.246^{ab}	3.628^{a}	43310^{b}	2464^{ab}	426.700^{ab}
WWM-33	0.019^{a}	2.075^{a}	15.130^{a}	427.600^{ab}	0.035^{a}	1.069^{ab}	869^{a}	7.293^{a}	482.300^{ab}	1576^{abcd}	0.693^{abcd}	0.085^{abc}	0.224^{ab}	3.263^{ab}	41857^{b}	2810^{ab}	369.100^{ab}
WWM-34	0.033^{a}	**4.119^{a}**	18.290^{a}	466^{ab}	0.039^{a}	1.165^{ab}	634.400^{a}	4.360^{a}	452.900^{ab}	1536^{abcd}	0.704^{abcd}	0.162^{abc}	0.246^{ab}	3.411^{a}	85871^{ab}	4815^{ab}	412.200^{ab}
WWM-35(1)	**0.016^{a}**	2.714^{a}	14.720^{a}	468.600^{ab}	0.031^{a}	1.171^{ab}	1034.700^{a}	5.458^{a}	484.300^{ab}	1182^{abcd}	0.590^{cd}	0.133^{abc}	**0.131^{b}**	2.441^{ab}	69008^{ab}	4554^{ab}	407.100^{ab}
WWM-35(2)	0.018^{a}	3.582^{a}	**19.410^{a}**	426.600^{ab}	0.045^{a}	1.066^{ab}	**1225.600^{a}**	5.432^{a}	489.800^{ab}	1504^{abcd}	0.673^{abcd}	0.120^{abc}	0.226^{ab}	3.084^{ab}	69950^{ab}	3661^{ab}	491.100^{ab}
WWM-37(2)	0.028^{a}	3.421^{a}	18.350^{a}	409^{ab}	0.045^{a}	1.022^{ab}	704.900^{a}	5.570^{a}	477^{ab}	1452^{absc}	0.670^{abcd}	0.113^{abc}	0.211^{ab}	2.830^{ab}	58693^{ab}	3305^{ab}	363.100^{ab}
WWM-38	0.044^{a}	2.751^{a}	16.130^{a}	431.700^{ab}	0.037^{a}	1.079^{ab}	847.300^{a}	5.865^{a}	501.700^{ab}	1212^{abcd}	0.569^{cd}	**0.260^{a}**	0.254^{ab}	3.312^{ab}	**138416^{a}**	8960^{ab}	552^{ab}
WWM-39	0.036^{a}	2.081^{a}	14.330^{a}	434.500^{ab}	0.033^{a}	1.086^{ab}	633.500^{a}	7.967^{a}	397.100^{b}	950^{abcd}	0.525	0.132^{abc}	0.292^{ab}	2.667^{ab}	65779^{ab}	4729^{ab}	440.300^{ab}
WWM-40	0.030^{a}	3.755^{a}	17.280^{a}	433.900^{ab}	0.040^{a}	1.085^{ab}	645.900^{a}	5.193^{a}	392.800^{b}	**781^{d}**	**0.497^{d}**	0.084	0.302^{ab}	2.658^{ab}	45463^{ab}	2614^{ab}	416.700^{ab}
WWM-41(A)	0.031^{a}	2.509^{a}	15.040^{a}	467.300^{ab}	0.032^{a}	1.168^{ab}	794.300^{a}	6.111^{a}	398^{b}	975^{abcd}	0.593^{cd}	0.214^{abc}	0.288^{ab}	2.476^{ab}	87847^{ab}	6406^{ab}	575.300^{ab}
WWM-41(B)	0.031^{a}	3.345^{a}	15.880^{a}	422.800^{ab}	0.038^{a}	1.057^{ab}	537.900^{a}	4.842^{a}	453.500^{ab}	1320^{abcd}	0.632^{bcd}	0.129^{abc}	0.212^{ab}	2.902^{ab}	**37711^{b}**	2395^{ab}	563.700^{ab}
WWM-44	0.026^{a}	3.033^{a}	13.880^{a}	443.800^{ab}	0.031	1.110^{ab}	612.600^{a}	4.765^{a}	428.100^{ab}	1442^{abcd}	0.826^{ab}	0.127^{abc}	0.299^{ab}	3.125^{ab}	67278^{ab}	4844^{ab}	362.700^{ab}
WWM-45(A)	0.034^{a}	2.528^{a}	14.070^{a}	446.300^{ab}	0.032^{a}	1.116^{ab}	651.700^{a}	5.906^{a}	446^{ab}	1250^{abcd}	0.639^{bcd}	0.226^{abc}	0.268^{ab}	3.017^{ab}	120407^{ab}	8620^{ab}	376.500^{ab}
WWM-45(B)	0.027^{a}	2.687^{a}	15.590^{a}	**393.400^{b}**	0.040^{a}	**0.983^{b}**	706.900^{a}	5.743^{a}	472.700^{ab}	1330^{abcd}	0.643^{bcd}	0.130^{abc}	0.210^{ab}	3.053^{ab}	69087	4476^{ab}	420^{ab}
WWM-46	0.038^{a}	3.543^{a}	15.890^{a}	445.900^{ab}	0.036^{a}	1.115^{ab}	543.500^{a}	4.516^{a}	451.800^{ab}	1668^{ab}	0.729^{abc}	0.177^{abc}	0.294^{ab}	**3.692^{a}**	94277^{ab}	6080^{ab}	406.800^{ab}
WWM-47	0.046^{a}	3.352^{a}	15.360^{a}	**471.600^{a}**	0.033^{a}	**1.179^{a}**	372.400^{a}	4.972^{a}	492^{b}	1738^{a}	0.717^{abc}	0.158^{abc}	0.247^{ab}	3.533^{a}	57159^{ab}	3736^{ab}	395.100^{ab}
WWM-50	0.032^{a}	2.984^{a}	14.840^{a}	464.300^{ab}	0.032^{a}	1.161^{ab}	471.600^{a}	5.399^{a}	443.800^{ab}	1239^{abcd}	0.609^{cd}	**0.052^{c}**	0.165^{ab}	2.723^{ab}	57370^{ab}	3990^{ab}	397.900^{ab}
WWM-57	**0.055^{a}**	2.803^{a}	**13.740^{a}**	446.300^{ab}	**0.031^{a}**	1.116^{ab}	306.800^{a}	4.993^{a}	458.400^{ab}	1145^{abcd}	0.606^{cd}	0.234^{abc}	0.272^{ab}	3.197^{ab}	124501	**9102^{a}**	448.600^{ab}

WWM-64	0.052^{a}	2.613 ^{a}	15.130^{a}	451.400^{ab}	0.034^{a}	1.128^{ab}	394.100^{a}	5.797 ^{a}	469.200^{ab}	1672^{a}	0.719^{abc}	0.180^{abc}	0.315 ^{ab}	3.569^{a}	71795^{ab}	4824^{ab}	459.700^{ab}
WWM-64(2)	0.037 ^{a}	3.903 ^{a}	14.630^{a}	442.700^{ab}	0.033^{a}	1.107^{ab}	432.300^{a}	**3.901 ^{a}**	**546.500^{a}**	1614^{abc}	0.658^{abcd}	0.127 ^{abc}	0.259 ^{ab}	2.948^{ab}	80198^{ab}	5578^{ab}	477.500^{ab}
WWM-65	0.036 ^{a}	1.848 ^{a}	14.720^{a}	406.300^{ab}	0.036^{a}	1.016^{ab}	616.900^{a}	8.363 ^{a}	459.500^{ab}	1316^{abcd}	0.649^{bcd}	0.126 ^{abc}	0.254 ^{ab}	2.862^{ab}	113084^{ab}	8148^{ab}	390.900^{ab}
WWM-66	0.027 ^{a}	2.614 ^{a}	14.990^{a}	421.600^{ab}	0.036^{a}	1.054^{ab}	588.500^{a}	5.962 ^{a}	447.100^{ab}	1248^{abcd}	0.640^{bcd}	0.090 ^{abc}	0.300 ^{ab}	3.121^{ab}	48927^{ab}	3271^{ab}	582^{ab}
WWM-67	0.022 ^{a}	3.915 ^{a}	16.040^{a}	455.100^{ab}	0.036^{a}	1.138^{ab}	762.300^{a}	4.193 ^{a}	410.900^{b}	1081^{abcd}	0.570^{cd}	0.112 ^{abc}	0.284 ^{ab}	3.539^{a}	75659^{ab}	4806^{ab}	453^{ab}
WWM-68	0.020^{a}	2.581 ^{a}	14.310^{a}	426.700^{ab}	0.034^{a}	1.067^{ab}	754.200^{a}	5.927 ^{a}	475.500^{ab}	843^{bcd}	0.671^{abcd}	0.129 ^{abc}	0.189 ^{ab}	3.349^{ab}	74589^{ab}	5561^{ab}	423.400^{ab}
WWM-76	0.032^{a}	3.553 ^{a}	19.210^{a}	427.400^{ab}	0.045	1.069^{ab}	722^{a}	6.307 ^{a}	472^{ab}	1467^{abcd}	0.678^{abcd}	0.143 ^{abc}	0.261 ^{ab}	3.108^{ab}	65218^{ab}	3444^{ab}	446.200^{ab}
WWM-79	0.026 ^{a}	2.735 ^{a}	17.380^{a}	409.800^{ab}	0.042^{a}	1.024^{ab}	680.400^{a}	6.382 ^{a}	465.700^{ab}	1303^{abcd}	0.638^{bcd}	0.102 ^{abc}	0.245 ^{ab}	2.776^{ab}	44724^{ab}	2721^{ab}	440.900^{ab}
WWM-81	0.028 ^{a}	3.411 ^{a}	19.080^{a}	460.700^{ab}	0.041^{a}	1.152^{ab}	679.600^{a}	5.703 ^{a}	416.800^{b}	1036^{abcd}	0.587^{cd}	0.121 ^{abc}	0.240 ^{ab}	2.967^{ab}	39494^{b}	**2157^{b}**	512^{ab}
LSD	0.030	1.443	5.574	38.350	0.014	0.096	553.900	3.134	60.840	407.300	0.105	0.090	0.092	0.627	46221.800	3401.600	150.920
CV (%)	60.800	29.900	21.1oo	5.400	22.600	5.400	49.500	32.400	8.200	19.200	9.900	42.100	23.100	12.800	41.600	47.500	20.900
P-value	0.877	0.134	0.925	<.001	0.721	<.001	0.734	0.317	<.001	<.001	<.001	<.001	0.016	<.001	0.001	<.001	0.011

Different upper-case letters within a column indicate significant difference among genotypes. ***gs***; (mmol m^{-2} s^{-1}), ***T***; (mmol H$_2$O m^{-2} s^{-1}), ***A***; (µmol CO$_2$ m^{-2} s^{-1}), ***A/Ci***; (µmol. mol m^{-1}), ***Ci***; (µmol. mol m^{-1}), ***WUEi***; [(µmol (CO$_2$) m^{-2}]; ***WUEins***, (µmol. mol^{-1}), ***Fv/Fm***; (ratio); ***ΦPSII***, the effective quantum efficiency of PSII photochemistry; ***qP***, photochemical quenching; ***qN***, non-photochemical quenching; ***ETR***, (µmol e^{-1} m^{-2} s^{-1}); ***ETR/A***, (µmol e µmol^{-1} CO$_2$); ***AES***, alternative electron sinks

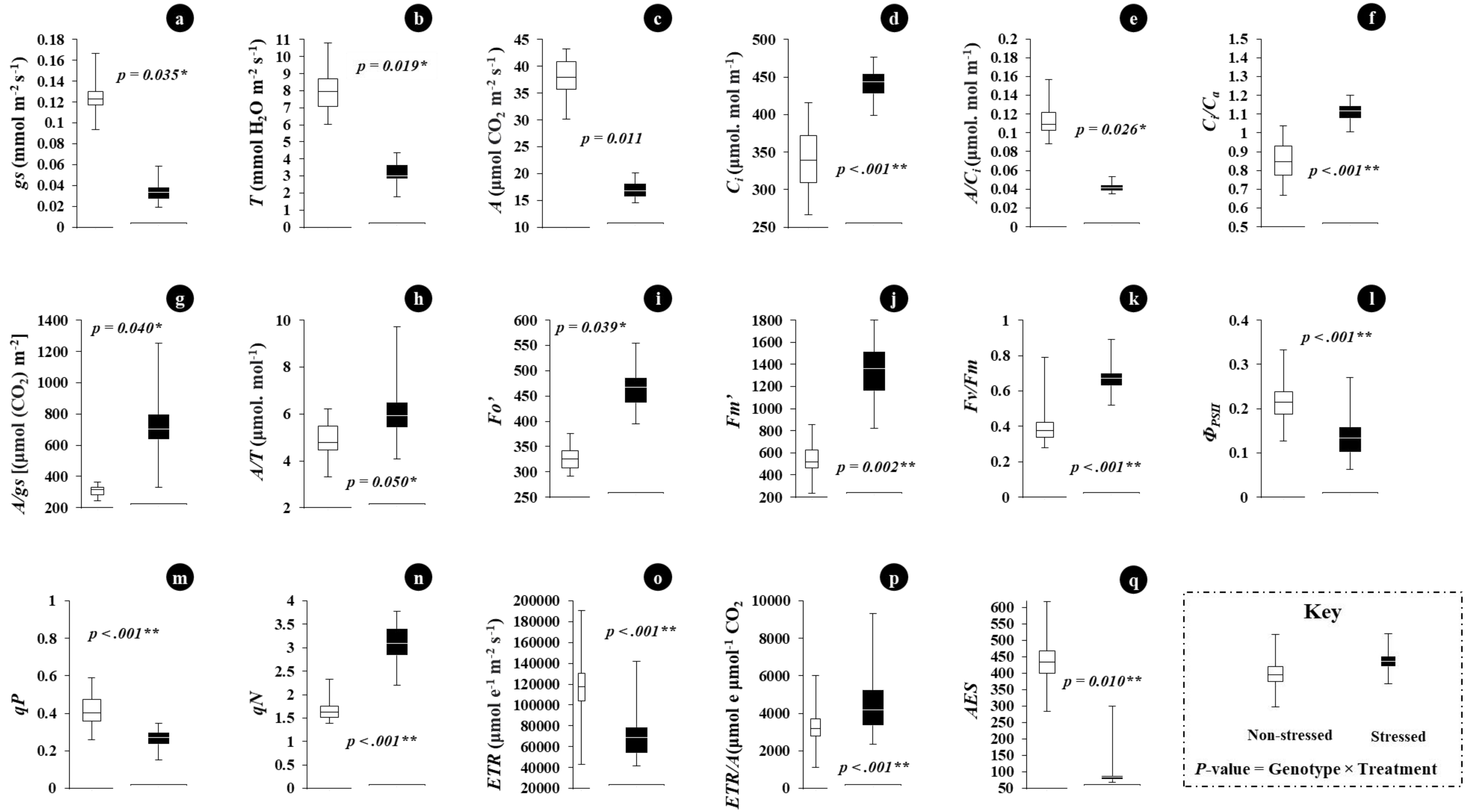


Figure 4.2: Effect of water stress in light adapted leaves of citron watermelon landrace accessions: (**a**) stomatal conductance (*gs*), (**b**) transpiration rate (*T*), (**c**) net CO_2 assimilation rate (*A*), (**d**) intercellular CO_2 concentration (C_i), (**e**) CO_2 assimilation rate/intercellular CO_2 concentration (A/C_i) (**f**) ratio of intercellular and atmospheric CO_2 (C_i/C_a), (**g**) intrinsic water use efficiency (*A/gs*), (**h**) instantaneous water-use efficiency (*A/T*), (**i**) minimum fluorescence (F_o'), (**j**) maximum fluorescence ($F_{m'}$), (**k**) maximum quantum efficiency of photosystem II photochemistry (F_v/F_m), (**l**) effective quantum efficiency of PSII photochemistry (Φ_{PSII}), (**m**) photochemical quenching (*qP*), (**n**) non-photochemical quenching (*qN*), (**o**) electron transport rate (*ETR*), (**p**) relative measure of electron transport to oxygen molecules (*ERT/A*), (**q**) alternative electron sink (*AES*)

4.3.2 Plant performance

ANOVA revealed accessions, irrigation regimes and their interactions were statistically significant for most morphological traits (Table 4.6). When compared to NS condition, DS significantly ($P < 0.05$) reduced *LA* and *SLA* among the accessions (Table 4.7). The evaluated accessions presented considerable variations in *RDM* under NS and DS conditions. *RDM* ranged between 0.529 and 2.764 g under NS condition, and 0.716 and 3.517 g under DS condition (Table 4.7). Accessions WWM-46, WWM-16, WWM-15 and WWM-21 recorded *RDM* ≥ 2.144 g under NS condition, whereas accessions WWM-50, WWM-15, WWM-35(2) and WWM-28 recorded *RDM* ≥ 3.039 g under DS conditions. Accessions WWM-24, WWM-81, WWM-35(1) and WWM-35(2) recorded *SLA* ≥ 288.300 g cm^{-2} under NS condition, whereas accessions WWM-08, WWM-24, WWM-35(1) and WWM-35(2) recorded *SLA* ≥ 242.500 g cm^{-2} under DS condition.

Taproot length of ≥ 84.32 cm was recorded for WWM-64, WWM-35(2) and WWM-76 under NS condition, whereas values for *TRL* ≥ 96.850 cm were recorded for WWM-50, WWM-64 and WWM-28 under DS condition. Highest *ERP* (≥ 82.20%) was recorded for accessions WWM-34, WWM-35(2), WWM-16, WWM-15 and WWM-50 under DS condition (Figure 4.3). The lowest ERP (≤ 53.83%) was recorded for WWM-14, WWM-38, WWM-09, WWM-07 and WWM-02 under DS condition (Table 4.7). Fruit yield ≥ 1.881 kg pot^{-1} was recorded for WWM-14, WWM-08, WWM-35(1) and WWM35(2) under NS condition, whereas fruit yield of ≥ 0.947 kg pot^{-1} were recorded for WWM-24 and WWM-81 under DS condition.

Table 4.6: Combined analysis of variance with mean squares and significant tests for leaf traits, root traits and yield in citron watermelon genotypes grown under drought-stress and non-stressed conditions

		Leaf parameters			**Root phenes**			**Yiel**
Source of variation	**d.f**	**LA**	**LDW**	**SLA**	**TRL**	**RDM**	**ERP**	**Yield**
Genotype	39	1.369	$2.862\times10^{-4**}$	1700.030^{**}	841.406^{**}	2.541^{**}	314.360^{**}	0.053^{**}
Irrigation	1	26.543^{**}	$9.762\times10^{-3**}$	99759.800^{**}	4147.314^{**}	19.487^{**}	4452.790^{**}	51.573
Genotype×Irrigation	39	2.181	$7.345\times10^{-5*}$	260.040^{**}	105.516^{**}	0.682^{**}	15.750^{*}	0.023^{**}
Residual	160	1.635	3.709×10^{-5}	33.470	4.809	0.037	10.250	0.007

d f; degrees of freedom, **LA**; leaf area, **LDW**; leaf dry weight, **SLA**, specific leaf area, **TRL**, taproot length, **RDM**; root dry mass, **ERP**; estimated rooting percentage

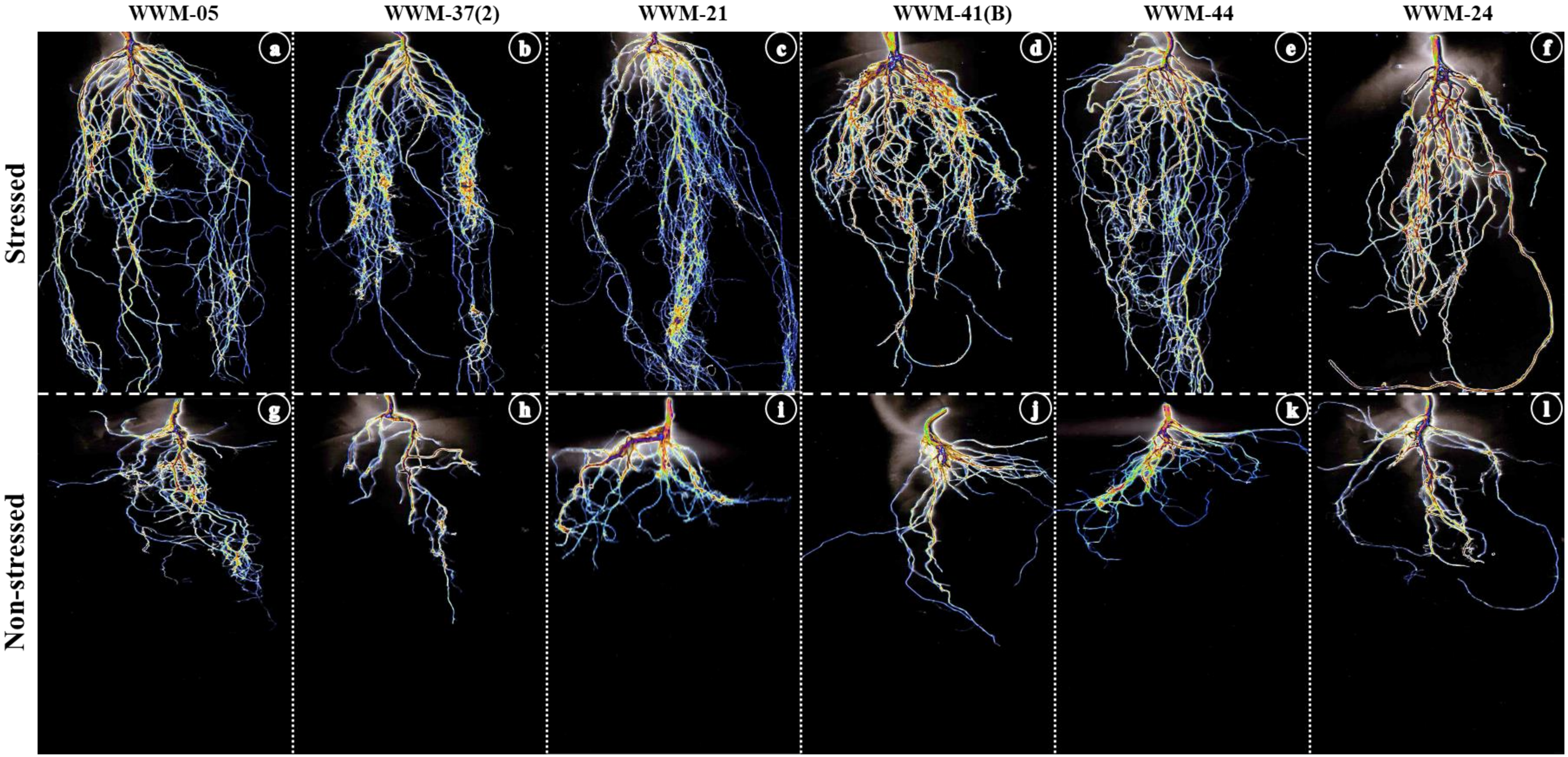


Figure 4.3: Visual aid of RootSnap analyses showing root morphology of selected citron watermelon accessions under drought-stressed and non-stressed conditions

Table 4.7: Mean values for leaf and root traits, and fruit yield of citron watermelon accessions evaluated under non-stressed and drought-stressed conditions

	LA (cm^{2})		**LDW (g)**		**SLA (g cm^{-2})**		**TRL (cm)**		**RDM (g)**		**ERP (%)**		**Fruit yield (kg/pot)**	
Accession	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**	**NS**	**DS**
WWM-02	22.140^{a}	20.550^{a}	0.093^{abcd}	0.104^{abcde}	237.900^{o}	197.400^{jklm}	54.300^{opq}	57.630^{mn}	0.535^{p}	0.832^{pqr}	**49.620^{r}**	**57.640^{l}**	1.462^{f}	0.760^{abc}
WWM-03	21.770^{a}	21.220^{a}	0.076^{efgh}	0.088^{bcdef}	287.500^{b}	241.400^{abcd}	78.830^{abcd}	87.530^{cd}	1.365^{fghijklm}	2.875^{abcdef}	70.940^{abcdefghi}	78.690^{abcdef}	1.865^{abcd}	0.815^{abc}
WWM-05	22.300^{a}	**22.640^{a}**	0.094^{abc}	**0.119^{a}**	236.400^{o}	**191.500^{m}**	63.930^{jklm}	69.630^{jk}	1.643^{defgh}	1.464^{jklmnopq}	60.250^{jklmnopq}	71.330^{bcdefghijk}	**1.432^{f}**	0.672^{bc}
WWM-07	22.140^{a}	21.590^{a}	0.089^{abcdefg}	0.095^{bcdef}	249.500^{hijk}	226.800^{bcdefgh}	52.020^{pq}	53.470^{n}	0.955^{klmnop}	0.740^{qr}	51.850^{qr}	63.830^{ijkl}	1.677^{cdef}	0.698^{bc}
WWM-08	22.640^{a}	**19.710^{a}**	0.079^{bcdefgh}	**0.081^{f}**	287.900^{b}	242.500^{abc}	71.320^{defghij}	75.670^{fghi}	1.820^{cdef}	1.359^{klmnopqr}	59.660^{klmnopq}	70.730^{cdefghijk}	1.884^{abc}	0.800^{abc}
WWM-09	20.730^{a}	21.550^{a}	0.077^{defgh}	0.101^{abcdef}	270^{c}	214.500^{efghijklm}	53.150^{opq}	65.600^{kl}	0.551^{op}	1.156^{lmnopqr}	53.380^{pqr}	64.910^{hijkl}	1.630^{cdef}	0.717^{bc}
WWM-14	22.240^{a}	22.050^{a}	0.078^{cdefgh}	0.100^{abcdef}	285.800^{b}	220.900^{cdefghijk}	68.230^{fghijk}	74.420^{fghij}	0.670^{nop}	**0.716^{r}**	53.830^{opqr}	62.030^{jkl}	1.881^{abc}	0.781^{abc}
WWM-15	21.820^{a}	20.880^{a}	0.091^{abcde}	0.105^{abcd}	239.300^{lmno}	199.400^{ijklm}	64.120^{jklm}	78.370^{ef}	2.597^{ab}	3.135^{abcd}	76.030^{ab}	82.830^{ab}	1.523^{ef}	0.701^{bc}
WWM-16	21.980^{a}	21.810^{a}	0.086^{abcdefgh}	0.100^{abcdef}	254.400^{fghi}	219.700^{cdefghijk}	76.350^{abcdef}	94.250^{b}	2.294^{abc}	3.038^{abcd}	72.63^{abcde}	82.640^{ab}	1.640^{cdef}	0.829^{abc}
WWM-17(2)	23.290^{a}	20.760^{a}	0.093^{abcd}	0.093^{bcdef}	250.500^{hijk}	223.300^{bcdefghi}	**51.220^{q}**	56.620^{mn}	0.602^{op}	0.905^{opqr}	59.730^{klmnopq}	60.030^{kl}	1.597^{cdef}	0.772^{abc}
WWM-21	20.470^{a}	20.880^{a}	0.072^{gh}	0.098^{abcdef}	283.200^{b}	214.100^{efghijklm}	58.570^{mnopq}	70.200^{hijk}	**2.764^{a}**	2.209^{efghi}	72.270^{abcdef}	82.350^{abc}	1.876^{abcd}	0.783^{abc}
WWM-23	21.910^{a}	20.970^{a}	0.086^{abcdefgh}	0.100^{abcdef}	254.500^{fghi}	210.300^{fghijklm}	74.420^{defg}	85.980^{cd}	1.402^{fghijklm}	2.713^{bcdefg}	67.450^{bcdefghijk}	77.140^{abcdefg}	1.676^{cdef}	0.819^{abc}
WWM-24	21.140^{a}	21.320^{a}	0.073^{fgh}	0.086^{cdef}	288.300^{b}	246.900^{ab}	77.430^{abcd}	94.200^{b}	1.583^{efghi}	3.058^{abcd}	65.010^{cdefghijklm}	80.910^{abcd}	1.735^{abcdef}	**0.955^{a}**
WWM-28	21.410^{a}	22.420^{a}	0.086^{abcdefgh}	0.100^{abcdef}	248.500^{hijklm}	224.400^{bcdefghi}	76.770^{abcde}	96.850^{b}	1.985^{cde}	3.141^{abc}	71.600^{abcdefg}	81.510^{abc}	1.723^{abcdef}	0.740^{bc}
WWM-33	22.270^{a}	20.500^{a}	0.083^{abcdefgh}	0.102^{abcdef}	267.300^{cde}	201^{ijklm}	54.070^{opq}	57.330^{mn}	0.654^{nop}	1.124^{lmnopqr}	55.57^{nopqr}	67.790^{fghijkl}	1.869^{abcd}	0.777^{abc}
WWM-34	21.800^{a}	20.980^{a}	0.085^{abcdefgh}	0.090^{bcdef}	255.600^{fghi}	232.600^{abcdef}	77.300^{abcde}	86.730^{cd}	1.730^{defg}	2.958^{abcd}	71.140^{abcdefgh}	82.200^{abc}	1.754^{abcdef}	0.661^{bc}
WWM-35(1)	21.600^{a}	20.970^{a}	**0.071^{h}**	0.082^{ef}	302.900^{a}	**256.200^{a}**	59.630^{lmnop}	**52.220^{n}**	**0.529^{p}**	0.843^{pqr}	57.080^{lmnopqr}	61.170^{jkl}	2.011^{ab}	0.844^{ab}
WWM-35(2)	23.260^{a}	20.820^{a}	0.076^{defgh}	0.081^{f}	**304.200^{a}**	255.900^{a}	84.420^{ab}	95.730^{b}	1.713^{defg}	**3.517^{a}**	72.170^{abcdef}	82.260^{abc}	**2.048^{a}**	0.824^{abc}
WWM-37(2)	21.430^{a}	21.030^{a}	0.090^{abcdef}	0.107^{abc}	237.400^{o}	196.200^{klm}	72.580^{defghi}	96.130^{b}	1.448^{fghijkl}	2.656^{bcdefg}	61.600^{jklmnop}	66.390^{ghijkl}	1.580^{cdef}	**0.632^{c}**
WWM-38	22.260^{a}	20.940^{a}	0.088^{abcdefgh}	0.095^{bcdef}	252.400^{ghij}	220.700^{cdefghijk}	65.420^{hijklm}	75.900^{fgh}	0.944^{lmnop}	1.137^{lmnopqr}	53.490^{pqr}	65.410^{ghijkl}	1.648^{cdef}	0.835^{ab}
WWM-39	20.040^{a}	21.110^{a}	0.075^{efgh}	0.102^{abcdef}	265.700^{cde}	206.400^{ghijklm}	65.900^{hijklm}	76.720^{efg}	1.232^{ghijklm}	1.500^{ijklmnop}	67.630^{abcdefghijk}	74.910^{abcdefghi}	1.573^{cdef}	0.707^{bc}
WWM-40	22.170^{a}	21.200^{a}	0.086^{abcdefgh}	0.095^{bcdef}	257.900^{efgh}	223.700^{bcdefghi}	54.750^{opq}	69.100^{jk}	1.164^{hijklmn}	0.908^{opqr}	56.280^{mnopqr}	65.640^{ghijkl}	1.709^{bcdef}	0.804^{abc}
WWM-41(A)	22.070^{a}	21.350^{a}	0.093^{abcde}	0.105^{abcd}	238.700^{no}	203.300^{hijklm}	73.070^{defgh}	87.370^{cd}	1.580^{efghi}	3.249^{ab}	**76.810^{a}**	79.640^{abcde}	1.572^{cdef}	0.698^{bc}
WWM-41(B)	21.230^{a}	20.870^{a}	0.087^{abcdefgh}	0.089^{bcdef}	244.300^{jklmno}	235.900^{abcde}	76.170^{bcdef}	82.200^{de}	1.389^{fghijklm}	2.475^{cdefgh}	62.770^{ghijklmno}	70.860^{cdefghijk}	1.752^{abcdef}	0.686^{bc}
WWM-44	20.280^{a}	21.060^{a}	0.077^{cdefgh}	0.091^{bcdef}	262.200^{cdef}	231.100^{abcdefg}	55.080^{opq}	77.970^{ef}	1.177^{hijklmn}	1.014^{mnopqr}	57.240^{lmnopqr}	69.170^{defghijkl}	1.748^{abcdef}	0.827^{abc}
WWM-45(A)	20.130^{a}	21.95	0.078^{bcdefgh}	0.103^{abcdef}	258^{efgh}	213.800^{efghijklm}	61.430^{klmno}	74.620^{fghij}	1.481^{efghijk}	1.628^{ijklmno}	62.470^{ghijklmnop}	71.840^{abcdefghij}	1.718^{abcdef}	0.795^{abc}
WWM-45(B)	23.640^{a}	20.690^{a}	**0.097^{a}**	0.104^{abcdef}	244.600^{jklmno}	200.500^{ijklm}	79.550^{abcd}	78.420^{ef}	1.081^{ijklmno}	2.413^{defgh}	64.210^{defghijklmn}	74.060^{abcdefghi}	1.633^{cdef}	0.808^{abc}
WWM-46	22.620^{a}	20.450^{a}	0.085^{abcdefgh}	0.096^{bcdef}	267.100^{cde}	214.100^{efghijklm}	75.550^{cdefg}	77.530^{efg}	2.144^{bcd}	1.841^{hijkl}	63.480^{efghijklmn}	64.830^{hijkl}	1.625^{cdef}	0.712^{bc}
WWM-47	21.970^{a}	21.560^{a}	0.092^{abcde}	0.107^{abcd}	239^{mno}	202^{hijklm}	65.120^{hijklm}	61.380^{lm}	1.511^{efghij}	1.597^{ijklmno}	61.750^{ijklmnop}	68.340^{efghijkl}	1.572^{cdef}	0.668^{bc}
WWM-50	20.140^{a}	21.270^{a}	0.076^{defgh}	0.091^{bcdef}	263.400^{cdef}	233.300^{abcdef}	83.730^{abc}	**103.220^{a}**	1.662^{defgh}	3.039^{abcd}	74.090^{abc}	**83.550^{a}**	1.697^{bcdef}	0.732^{bc}
WWM-57	22.430^{a}	20.480^{a}	0.084^{abcdefgh}	0.099^{abcdef}	268.600^{cd}	206.700^{ghijklm}	64.600^{ijklm}	77.480^{efg}	1.473^{efghijkl}	1.476^{jklmnop}	68.210^{abcdefghijk}	72.930^{abcdefghij}	1.568^{cdef}	0.731^{bc}
WWM-64	21.140^{a}	21.030^{a}	0.088^{abcdefgh}	0.105^{abcd}	241.200^{klmno}	200.800^{ijklm}	84.320^{ab}	103.080^{a}	1.57^{efghi}	2.936^{abcde}	69.210^{abcdefghij}	81.990^{abc}	1.546^{def}	0.716^{bc}
WWM-64(2)	21.400^{a}	19.730	0.086^{abcdefgh}	0.084^{def}	248.900^{hijkl}	234.200^{abcdef}	61.370^{klmno}	54.670^{n}	0.557^{op}	1.007^{mnopqr}	55.780^{nopqr}	61.720^{jkl}	1.618^{cdef}	0.828^{abc}
WWM-65	20.380^{a}	20.680^{a}	0/078^{bcdefgh}	0.094^{bcdef}	260.200^{defg}	220.500^{cdefghijk}	63.650^{jklmn}	56.480^{mn}	1.035^{jklmnop}	1.653^{ijklmn}	62.850^{ghijklmno}	72.790^{abcdefghij}	1.665^{cdef}	0.687^{bc}
WWM-66	21.320^{a}	21.190^{a}	0.080^{abcdefgh}	0.096^{abcdef}	267.300^{cde}	220.800^{cdefghijk}	55.370^{nopq}	64.700^{kl}	0.942^{lmnop}	1.348^{klmnopqr}	66.290^{hijklmnop}	66.870^{ghijkl}	1.618^{cdef}	0.740^{bc}
WWM-67	22.200^{a}	19.800^{a}	0.084^{abcdefgh}	0.089^{bcdef}	263^{cdef}	221.800^{bcdefghij}	67.580^{ghijkl}	69.830^{ijk}	1.771^{cdef}	2.019^{ghijk}	65.640^{cdefghijkl}	76.550^{abcdefgh}	1.806^{abcde}	0.817^{abc}
WWM-68	21.320^{a}	21.740^{a}	0.086^{abcdefgh}	0.100^{abcdef}	248.100^{ijklmn}	216.900^{defghijkl}	68.950^{efghijk}	85.670^{cd}	0.899^{mnop}	2.177^{fghij}	67.450^{bcdefghijk}	75.040^{abcdefghi}	1.611^{cdef}	0.737^{bc}
WWM-76	22.780	20.520^{a}	0.088^{abcdefgh}	0.095^{bcdef}	258.100^{efgh}	217.600^{cdefghijkl}	**84.720^{a}**	91.020^{bc}	1.412^{fghijklm}	2.941^{abcd}	73.240^{abcd}	80.180^{abcd}	1.818^{abcde}	0.747^{bc}

WWM-79	22.400^{a}	21.300^{a}	0.095^{ab}	0.110^{ab}	235.800^{o}	193.800^{lm}	75.530^{cdefg}	71.750^{ghij}	1.541^{efghij}	1.678^{ijklm}	63.260^{fghijklmn}	71.500^{bcdefghijk}	1.448^{f}	0.720^{bc}
WWM-81	21.510^{a}	21.550^{a}	0.074^{fgh}	0.085^{cdef}	291.700^{b}	253.300^{a}	58.130^{mnopq}	53.530^{n}	1.220^{ghijklm}	0.950^{nopqr}	56.170^{mnopqr}	66.490^{ghijkl}	1.797^{abcde}	0.947^{a}
l.SD	2.160	1.992	0.008	0.011	4.733	12.422	4.124	2.896	0.263	0.358	4.534	5.793	0.163	0.098
CV %	6.100	5.800	6.200	7.100	1.100	3.500	3.700	2.300	11.900	11.400	4.400	4.900	5.900	7.900
P-value	0.145	0.769	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

*Different upper-case letters within a column indicate significant difference among genotypes. Bolded figures represent highest and lowest values of each parameter in a column

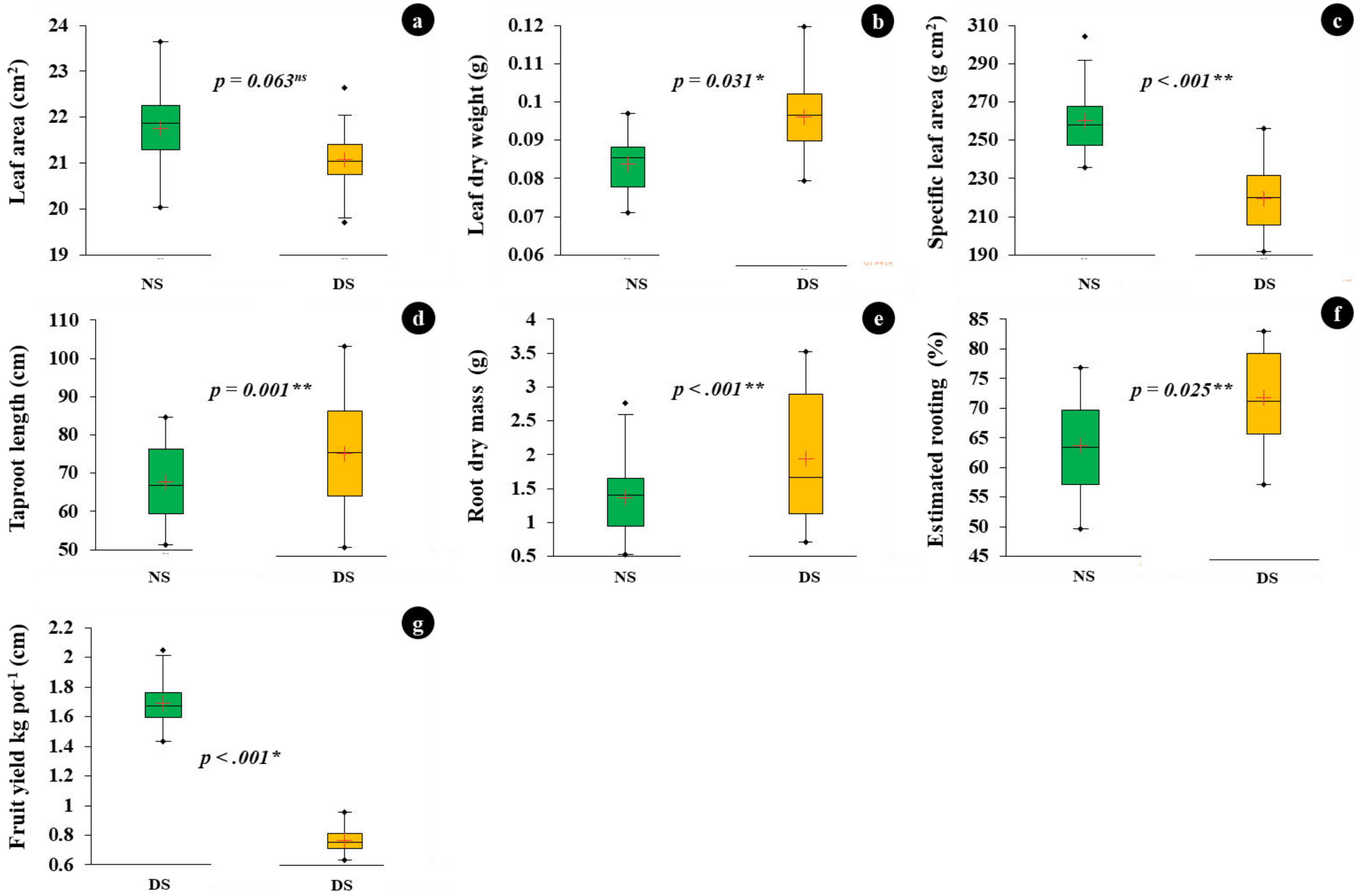


Figure 4.4: Response of citron watermelon accessions for morphological traits under non-stress and drought-stressed conditions.: (**a**) leaf area, (**b**) leaf dry mass, (**c**) specific leaf area, (**d**) taproot length, (**e**) root dry mass, (**f**) estimated rooting percentage (**g**) fruit yield

4.3.3 Genotypic classification for drought tolerance based on drought tolerance indices

To identify drought-tolerant and drought-sensitive genotypes, six drought tolerance indices (TOL, MPI, STI, GMP, YI and YSI) were calculated based on fruit yield of each accession under NS and DS conditions (Table 4.8). Higher *TOL* values ≥ 1.100 were recorded for WWM-14, WWM-35(2) and WWM-35(1), whereas lower *TOL* values ≤ 0.760 were recorded for WWM-05, WWM-79 and WWM-02. Accessions WWM-81, WWM-35(1) and WWM-35(2) recorded *MPI* values of ≥ 1.372, whereas *MPI* values ≤ 1.106 were recorded for WWM-05, WWM-37(2) and WWM-79. Accessions WWM-81, WWM-24 and WWM-35(2) recorded *STI* ≥ 0.665, whereas *STI* values ≤ 0.410 were recorded in WWM-05, WWM-37(2) and WWM-47. Accessions WWM-81, WWM-35(1) and WWM-35(2) recorded *GMP* ≥ 1.294, whereas *GMP* values ≤ 1.020 were recorded for WWM-05, WWM-37(2) and WWM-79. Higher *YI* values ≥ 1.107 were recorded in WWM-24, WWM-35(1) and WWM-81, whereas lower *YI* values ≤ 0.876 were recorded for WWM-37(2), WWM-47 and WWM-34.

Based on drought-tolerant indices, the accessions were classified into five groups (Figure 4.5). Group A (highly drought-tolerant) comprised of 14 genotypes (WWM-02, WWM-05, WWM-09, WWM-15, WWM-37(2), WWM-39, WWM-41(A), WWM-46, WWM-47, WWM-57, WWM-64, WWM-66, WWM-68 and WWM-79) with the highest fruit yield (≥ 1.358 kg pot^{-1}) and values for various drought tolerance indices.

Group B comprised of 9 genotypes (WWM-03, WWM-08, WWM-14, WWM-21, WWM-33, WWM-35(1), WWM-35(2), WWM-67 and WWM-76) with high tolerance indices values; hence, they are considered drought-tolerant genotypes.

Similarly, group C had 6 genotypes (WWM-07, WWM-28, WWM-34, WWM-41(B) with intermediate values of tolerance indices and fruit yield (≥ 1.347 kg pot^{-1}) and are considered moderately tolerant. Groups D and E contained 9 (WWM-16, WWM-17(2), WWM-23, WWM-38, WWM-40, WWM-44, WWM-45(A) and 2 (WWM-24 and WWM-81) accessions, respectively with low fruit yield and tolerance indices and are referred to as sensitive and highly sensitive genotypes in that order (Figure 4.5).

Table 4.8: Drought stress tolerance indices of 40 citron watermelon accessions evaluated under non-stressed and drought-stressed conditions

Accession	**Y_P (kg pot^{-1})**	**Y_s (kg pot^{-1})**	**TOL**	**MPI**	**STI**	**GMP**	**YI**	**YSI**
WWM-02	1.462 (38)	0.7602 (20)	**0.7022 (40)**	1.111 (37)	0.4399 (32)	1.053 (32)	0.996(20)	0.5204(3)
WWM-03	1.865 (7)	0.8145 (11)	1.0502 (10)	1.340 (6)	0.6065 (6)	1.231 (5)	1.067(11)	0.4392(24)
WWM-05	**1.432 (40)**	0.6717 (37)	0.7602 (38)	**1.052 (40)**	**0.3789 (40)**	**0.979 (40)**	0.880(37)	0.4710(13)
WWM-07	1.677 (19)	0.6982 (33)	0.9792 (13)	1.188 (24)	0.4511 (30)	1.082 (26)	0.916(33)	0.4173(33)
WWM-08	1.884 (3)	0.8005 (14)	1.0833 (7)	1.342 (5)	0.6066 (5)	1.228 (6)	1.048(14)	0.4248(29)
WWM-09	1.630 (25)	0.7168 (28)	0.9128 (20)	1.173 (29)	0.4519 (29)	1.079 (27)	0.940(28)	0.4418(23)
WWM-14	1.881 (4)	0.7813 (17)	1.1002 (3)	1.331 (7)	0.5649 (11)	1.210 (9)	1.025(17)	0.4205(30)
WWM-15	1.523 (37)	0.7007 (32)	0.8225 (33)	1.112 (36)	0.4167 (37)	1.033 (36)	0.919(32)	0.4604(17)
WWM-16	1.640 (23)	0.8292 (5)	0.8110 (35)	1.235 (17)	0.5284 (17)	1.166 (16)	1.088(5)	0.5057(6)
WWM-17(2)	1.597 (30)	0.7722 (19)	0.8252 (32)	1.185 (25)	0.4867 (22)	1.110 (22)	1.012(19)	0.4844(10)
WWM-21	1.876 (5)	0.7833 (16)	1.0927 (5)	1.330 (8)	0.5770 (8)	1.212 (8)	1.026(16)	0.4182(32)
WWM-23	1.676 (20)	0.8188 (9)	0.8568 (27)	1.247 (15)	0.5289 (16)	1.171 (13)	1.075(9)	0.4893(9)
WWM-24	1.735 (14)	**0.9548 (1)**	0.7798 (37)	1.345 (4)	0.6658 (3)	1.287 (4)	**1.251(1)**	**0.5508(1)**
WWM-28	1.723 (15)	0.7397 (23)	0.9835 (12)	1.231 (18)	0.4944 (21)	1.128 (20)	0.970(22)	0.4307(27)
WWM-33	1.869 (6)	0.7770 (18)	1.0923 (6)	1.323 (9)	0.5683 (10)	1.203 (10)	1.018(18)	0.4164(34)
WWM-34	1.754 (11)	0.6608 (39)	1.0933 (4)	1.208 (23)	0.4526 (28)	1.076 (28)	0.866(39)	**0.3769(40)**
WWM-35(1)	2.011 (2)	0.8437 (3)	1.1670 (2)	1.427 (2)	0.6583 (4)	1.302 (2)	1.107(3)	0.4193(31)
WWM-35(2)	**2.048 (1)**	0.8242 (8)	**1.2242 (1)**	**1.436 (1)**	0.6699 (2)	1.294 (3)	1.081(8)	0.4115(36)
WWM-37(2)	1.580 (31)	**0.6318 (40)**	0.9485 (16)	1.106 (38)	0.3924 (39)	0.999 (39)	**0.828(40)**	0.4000(38)
WWM-38	1.648 (22)	0.8345 (4)	0.8138 (34)	1.241 (16)	0.5405 (15)	1.173 (12)	1.095(4)	0.5061(5)
WWM-39	1.573 (32)	0.7068 (31)	0.8657 (26)	1.140 (32)	0.4307 (35)	1.053 (33)	0.927(31)	0.4514(21)
WWM-40	1.708 (17)	0.8037 (13)	0.9048 (21)	1.256 (14)	0.5476 (12)	1.170 (14)	1.052(13)	0.4725(12)
WWM-41(A)	1.572 (33)	0.6975 (34)	0.8748 (24)	1.135 (33)	0.4343 (34)	1.046 (35)	0.914(34)	0.4441(22)
WWM-41(B)	1.752 (12)	0.6857 (36)	1.0660 (9)	1.219 (21)	0.4693 (24)	1.096 (23)	0.899(36)	0.3916(39)
WWM-44	1.748 (13)	0.8270 (7)	0.9213 (18)	1.288 (11)	0.5709 (9)	1.202 (11)	1.084(7)	0.4736(11)
WWM-45(A)	1.718 (16)	0.7952 (15)	0.9232 (17)	1.257 (13)	0.5468 (13)	1.168 (15)	1.041(15)	0.4621(16)
WWM-45(B)	1.633 (24)	0.8077 (12)	0.8255 (31)	1.220 (20)	0.5125 (19)	1.148 (19)	1.060(12)	0.4954(8)
WWM-46	1.625 (26)	0.7118 (30)	0.9132 (19)	1.168 (30)	0.4592 (26)	1.073 (29)	0.932(30)	0.4376(25)
WWM-47	1.572 (34)	0.6680 (38)	0.9038 (22)	1.120 (35)	0.4100 (38)	1.024 (37)	0.876(38)	0.4252(28)
WWM-50	1.697 (18)	0.7323 (25)	0.9647 (15)	1.215 (22)	0.5007 (20)	1.114 (21)	0.958(26)	0.4307(26)

WWM-57	1.568(35)	0.7313 (26)	0.8363 (29)	1.149 (31)	0.4529 (27)	1.070 (30)	0.959(25)	0.4684(14)
WWM-64	1.546 (36)	0.7157 (29)	0.8300 (30)	1.131 (34)	0.4376 (33)	1.051 (34)	0.937(29)	0.4631(15)
WWM-64(2)	1.618 (28)	0.8283 (6)	0.7895 (36)	1.223 (19)	0.5239 (18)	1.157 (18)	1.087(6)	0.5134(4)
WWM-65	1.665 (21)	0.6873 (35)	0.9778 (14)	1.176 (27)	0.4508 (31)	1.069 (31)	0.900(35)	0.4137(35)
WWM-66	1.618 (27)	0.7400 (22)	0.8780 (23)	1.179 (26)	0.4723 (23)	1.094 (24)	0.970(23)	0.4568(19)
WWM-67	1.806 (9)	0.8170 (10)	0.9892 (11)	1.312 (10)	0.5947 (7)	1.212 (7)	1.069(10)	0.4519(20)
WWM-68	1.611 (29)	0.7370 (24)	0.8735 (25)	1.174 (28)	0.4677 (25)	1.089 (25)	0.966(24)	0.4581(18)
WWM-76	1.817 (8)	0.7468 (21)	1.0707 (8)	1.282 (12)	0.5457 (14)	1.164 (17)	0.977(21)	0.4106(37)
WWM-79	1.448 (39)	0.7198 (27)	0.7280 (39)	1.084 (39)	0.4220 (36)	1.020 (38)	0.942(27)	0.4969(7)
WWM-81	1.797 (10)	0.9468 (2)	0.8502 (28)	1.372 (3)	**0.6710 (1)**	**1.303 (1)**	1.242(2)	0.5272(2)
LSD	0.128	0.078	0.130	0.084	0.186	0.081	0.097	0.047
CV%	6.600	9.000	12.300	6.000	32.000	6.300	8.500	9.200
P-value	<.001	<.001	<.001	<.001	0.058	<.001	<.001	<.001

The numbers in the parentheses are the genotype ranks for each index—**Y_p**; yield under well-watered condition, **Y_s**; yield under drought conditions, **SSI**; Stress Susceptibility Index, **TOL**; Tolerance, **MPI**; Mean Productivity Index, **STI**; Stress Tolerance Index, **GMP**; Geometric Mean Productivity, **YI**; Yield Index, **YSI**; Yield Stability Index, **DRI**; Drought Resistance Index

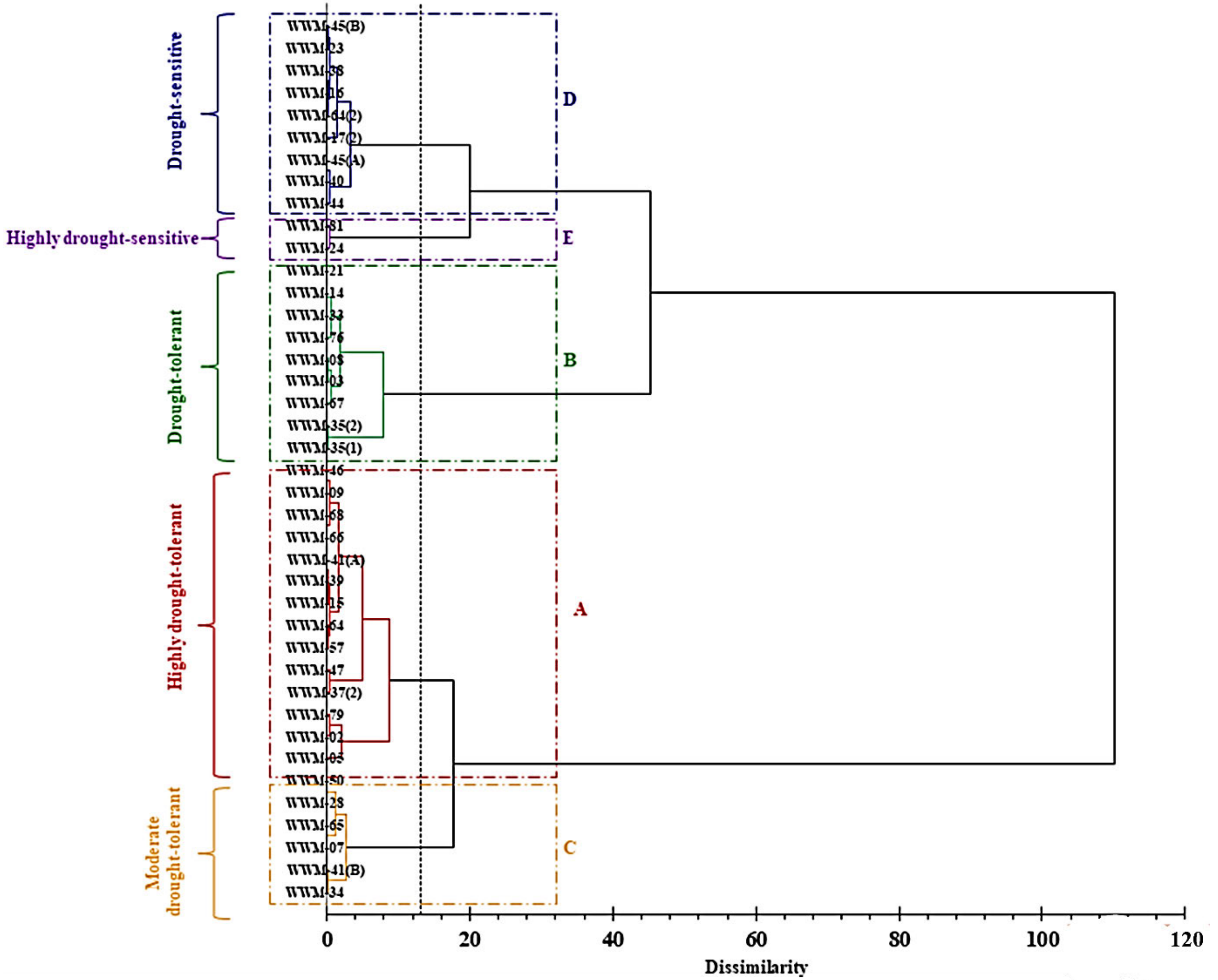


Figure 4.5: Dendrogram distinguishing the levels of drought tolerance among 40 citron watermelon accessions based on fruit yield under non-stressed and drought-stressed conditions

4.3.4 Principal component analysis (PCA) for assessed traits

Table 4.9 shows the PCA with factor loadings, eigenvalues, and percent variance for the evaluated traits. Under NS condition, PC1 accounted for 40.57% of the total variation and was positively correlated with *SLA*, fruit yield, *MPI* and *GMP*. PC2 positively correlated with Φ_{PSII}, *ETR*, *ETR/A* and *ERP*, contributing to 33.40% of the total variation. PC3 accounted for 12.82% of the total variation and was positively correlated with *A*, C_i, and C_i/C_a. WUE_{inst} was positively correlated with PC4, which accounted for 8.58% of the total variation. A/C_i and F_o' were positively correlated with PC5, which accounted for 4.63% of the total variation

Under DS condition, PC1 accounted for 47.32% of the total variation and was positively correlated with WUE_i, *SLA*, fruit yield, *MPI*, *STI*, *GMP* and *YI*. Net CO_2 assimilation rate, WUE_i and WUE_{inst} were positively correlated with PC2, accounting for 23.68% of the total variation. PC3 accounted for 12.33% of the total variation and positively correlated with F_m', F_v'/F_m', *qN*, TRL and RDM. Instantaneous water-use efficiency, ERP, TRL and RDM were positively correlated with PC4, which accounted for 9.26% of the total variation Transpiration rate and *qP* were positively correlated with PC, which accounted for 7.40% of the total variation (Table 4.9).

The PC biplots based on PCA analysis were used to picture the relationship among citron watermelon landraces based on evaluated physiological and morphological parameters under NS (Figure 4.6a) and DS conditions (Figure 4.6b). Traits represented by parallel vectors or close to each other revealed a strong positive association, and those located nearly opposite (at 180°) showed highly negative association, while the vectors toward sides expressed a weak relationship.

Under NS condition, accessions WWM-79, WWM-17(2), WWM-64(2) and WWM-45(B) are grouped based on high WUE_i, WUE_{inst} and *YSI*. Accessions WWM-08, WWM-14, WWM-21 and WWM35(2) are grouped based on high fruit yield, *GMP*, *TOL* and *MPI*. WWM-57, WWM-07, WWM-68 and WWM-28 are grouped based on high *qN*, *qP*, Φ_{PSII}, *RDM* and *LA*. Accessions WWM-44, WWM-45(A), WWM-66, WWM-35(1) and WWM-09 are grouped based on high *YI*

Under DS conditions, accessions WWM-79, WWM-02, WWM-68 and WWM-66 are grouped based on high WUE_{inst}, *LA* and *RDM*. Accessions WWM-76, WWM-33, WWM-21 and WWM-07 are grouped based on high WUE_i, *A* and *TOL*. WWM-46, WWM-45(A), WWM-38 and

WWM-41(A) are grouped based on high *gs*, *qP*, *ETR/A*, *qN* and Φ_{PSII}. Accessions WWM-44, WWM-67, WWM-14 and WWM-81 are grouped based on high C_i/C_a, *AES*, *T* and fruit yield.

4.3.5 Pearson correlation analysis

Pearson correlation coefficients showing relationships between physiological and morphological traits evaluated among citron watermelon accessions under NS and DS conditions are presented in (Table 4.10). Under NS condition, significant and positive correlations were observed between fruit yield and WUE_i (r = 0.69; p = 0.004), F_o' (r = 0.65; p = 0.04), F_v'/F_m' (r = 0.61; p = 0.007) and *qN* (r = 0.50; p = 0.048). Significant and negative correlations were observed between fruit yield and *gs* (r = -0.87; p = 0.02) and SLA (r = -0.54; p = 0.035). WUE_{inst} was negatively correlated with *ERP* (r = -0.85; p = 0.042) and *TRL* (r = -0.94; p = 0.025) (Table 4.10). Taproot length was positively correlated with *T* (r = 0.72; p = 0.036), C_i (r = 0.86; p = 0.004), C_i/C_a (r = 0.86; p = 0.038) and *AES* (r = 0.71; p = 0.049). The ERP was positively correlated with *T* (r = 0.76; p = 0.037), F_o' (r = 0.97; p = 0.011), *TRL* (r = 0.73; p = 0.029) and *RDM* (r = 0.65; p = 0.040).

Under DS condition, significant and positive correlations were observed between fruit yield and WUE_{inst} (r = 0.97; p = 0.013) and *LDW* (r = 0.77; p = 0.014). ERP was positively correlated with *qN* (r = 0.60; p = 0.037), *RDM* (r = 0.94; p = 0.011) and F_m' (r = 0.66; p = 0.025). RDM was positively correlated with *qN* (r = 0.81; p = 0.019) and *TRL* (r = 0.79; p = 0.038). Significant and negative correlations were observed between fruit yield and T (r = -0.81; p = 0.02) and *SLA* (r = -0.74; p = 0.032). The ERP was negatively correlated with Φ_{PSII} (r = -0.89; p = 0.004), *qP* (r =-0.96; p = 0.008) and *ETR/A* (r = -0.62; p = 0.042). RDM was negatively correlated with Φ_{PSII} (r = -0.80; p = 0.014) and *qP* (r = -0.88; p = 0.009). The *SLA* was negatively correlated with *ETR* (r = -0.83; p =0.032), *ETR/A* (r = -0.90; p = 0.027) and *LDW* (r = -0.98; p =0.021) (Table 4.10)

Table 4.9: Summary of factor loadings, eigenvalue, Kaiser-Meyer-Olkin measure of sampling adequacy, percent and cumulative variation for physiological parameters, leaf growth traits and root phenes assessed among 40 citron watermelon accessions under non-stressed and drought-stressed conditions

	Non-stressed						**Drought-stressed**					
Traits	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**KMO**	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**KMO**
gs	0.151	0.034	0.184	0.154	-0.137	0.345	-0.504	-0.584	0.300	0.046	0.209	0.502
T	0.029	0.067	0.082	-0.394	0.198	0.209	0.481	-0.019	0.362	-0.400	0.412	0.579
A	0.016	-0.184	0.340	0.257	0.112	0.425	0.456	0.501	0.180	-0.053	0.365	0.527
C_i	0.058	0.125	0.356	-0.057	-0.371	0.546	0.231	-0.619	0.231	0.271	0.003	0.556
A/C_i	-0.033	-0.249	-0.086	0.237	0.431	0.291	0.253	0.716	0.054	-0.183	0.314	0.515
C_i/C_a	0.058	0.125	0.356	-0.057	-0.371	0.545	0.231	-0.620	0.231	0.271	0.004	0.555
WUE_i	-0.113	-0.198	0.138	0.086	0.203	0.457	0.566	0.427	-0.192	-0.030	-0.324	0.528
WUE_{inst}	-0.010	-0.176	0.112	0.485	-0.088	0.250	-0.342	0.347	-0.343	0.433	-0.306	0.551
F_o'	-0.094	0.232	0.075	0.130	0.295	0.225	0.141	-0.071	0.428	-0.573	-0.007	0.441
F_m'	-0.148	0.059	0.204	0.187	0.031	0.193	0.082	0.054	0.751	-0.315	0.180	0.428
F_v/F_m	-0.137	0.286	0.039	0.122	0.113	0.572	0.014	0.085	0.675	-0.066	0.066	0.401
Φ_{PSII}	-0.145	0.308	-0.208	0.149	-0.060	0.724	-0.229	-0.620	0.213	-0.028	-0.128	0.487
qP	-0.070	0.097	-0.242	0.060	0.140	0.407	-0.226	-0.374	0.126	-0.003	0.406	0.386
qN	-0.204	0.128	0.167	0.190	0.201	0.418	-0.046	-0.066	0.602	0.022	0.197	0.584
ETR	-0.150	0.316	-0.222	0.125	-0.071	0.324	-0.274	-0.636	0.130	-0.108	-0.346	0.473
ETR/A	-0.131	0.322	-0.301	-0.002	-0.084	0.337	-0.372	-0.673	0.054	-0.034	-0.392	0.502
AES	-0.056	0.215	0.173	0.045	0.088	0.177	0.203	-0.152	0.112	-0.134	0.089	0.234
LA	-0.039	0.047	-0.105	-0.097	-0.109	0.097	-0.221	0.206	-0.007	0.280	0.032	0.164
LDW	-0.293	-0.071	-0.027	-0.118	-0.103	0.312	-0.833	0.231	-0.107	0.094	0.126	0.534
SLA	0.325	0.112	-0.041	0.080	0.056	0.342	0.861	-0.153	0.107	-0.001	-0.125	0.551
TRL	-0.034	0.236	0.117	0.160	0.009	0.457	-0.048	0.127	0.565	0.655	0.083	0.409
RDM	-0.035	0.166	0.053	0.186	-0.029	0.121	-0.014	0.288	0.525	0.698	0.011	0.428
ERP	-0.050	0.234	0.116	0.189	-0.068	0.190	0.012	0.239	0.450	0.753	-0.023	0.367
Fruit yield	0.330	0.161	0.045	-0.036	0.159	0.682	0.767	-0.326	-0.321	0.220	0.246	0.671
TOL	0.222	0.213	0.165	-0.169	0.261	0.467	0.533	0.246	0.430	-0.141	-0.593	0.422
MPI	0.354	0.104	-0.042	0.058	0.069	0.419	0.939	-0.084	0.042	0.073	-0.208	0.592
STI	0.345	0.058	-0.110	0.127	0.013	0.382	0.938	-0.166	-0.077	0.143	-0.053	0.622
GMP	0.348	0.057	-0.097	0.118	0.004	0.418	0.935	-0.172	-0.079	0.126	-0.072	0.757
YI	0.271	-0.050	-0.199	0.225	-0.139	0.368	0.767	-0.327	-0.321	0.217	0.244	0.688
YSI	-0.020	-0.197	-0.260	0.255	-0.293	0.403	0.023	-0.370	-0.544	0.232	0.608	0.524
Eigenvalue	6.980	4.512	3.845	2.575	2.256	-	7.274	4.351	3.700	2.778	2.116	-
Variability (%)	40.571	33.401	12.816	8.583	4.629	-	47.321	23.683	12.332	9.261	7.403	-
Cumulative (%)	40.571	73.972	86.788	95.371	100	-	47.321	71.004	83.336	92.597	100	-

gs; stomatal conductance; ***T***, transpiration rate, ***A***; net CO_2 assimilation rate; A/C_i, CO_2 assimilation rate/intercellular CO_2 concentration; C_i, intercellular CO_2 concentration; C_i/C_a, ratio of intercellular and atmospheric CO_2; WUE_i, intrinsic water use efficiency; WUE_{inst}, instantaneous water-use efficiency; F_v/F_m, maximum quantum efficiency of photosystem II photochemistry; Φ_{PSII}, the effective quantum efficiency of PSII photochemistry; ***qP***, photochemical quenching; ***qN***, non-photochemical quenching; ***ETR***, electron transport rate; ***ETR/A***, relative measure of electron transport to oxygen molecules; ***AES***, alternative electron sinks, ***LA***; leaf area, ***LDW***; leaf dry weight, ***SLA***; specific leaf area, ***TRL***; taproot length, ***RDM***; root dry mass, ***ERP***; estimated rooting percentage

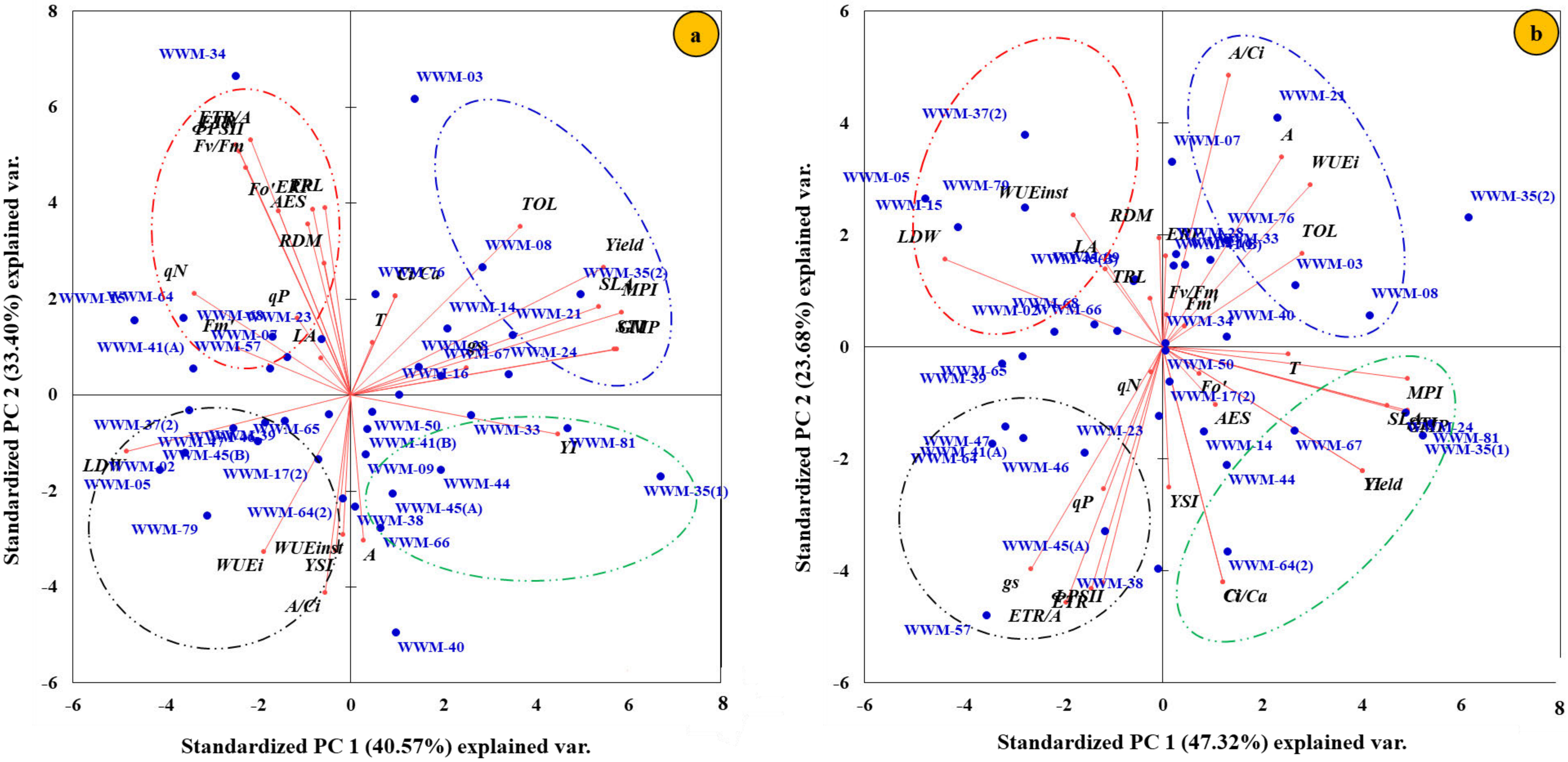


Figure 4.6: Principal component (PC) biplot of PC 1 vs PC 2 demonstrating the relationships among morphological and physiological traits among 40 citron watermelon accessions evaluated under (**a**) non-stressed conditions (**b**) drought-stressed conditions

Table 4.10: Pearson correlation coefficients for evaluated traits (gas exchange parameters, chlorophyll fluorescence parameters, leaf parameters, root phenes and yield) under non-stressed condition (*bottom diagonal*) and drought-stressed condition (*top diagonal*)

Traits	*gs*	*T*	*A*	C_i	A/C_i	C_i/C_a	WUE_i	WUE_{inst}	F_o'	F_m'	F_v/F_m	Φ_{PSII}	*qP*	*qN*	*ETR*	*ETR/A*	*AES*	*LA*	*LDW*	*SLA*	*TRL*	*RDM*	*ERP*	Yield
gs	1.00	-0.06	-0.26^{ns}	0.23^{ns}	-0.32^{ns}	0.23^{ns}	**-0.76****	-0.06^{ns}	0.10^{ns}	0.15^{ns}	0.02^{ns}	**0.61***	**0.44***	0.22^{ns}	**0.53***	**0.54***	-1E-3^{ns}	-0.07^{ns}	0.23^{ns}	-0.31^{ns}	0.16^{ns}	0.06^{ns}	0.07^{ns}	-0.22^{ns}
T	0.02^{ns}	1.00	**0.53***	0.20^{ns}	0.38^{ns}	0.20^{ns}	0.06^{ns}	**-0.82****	0.26^{ns}	0.30^{ns}	0.05^{ns}	-0.05^{ns}	0.08^{ns}	0.15^{ns}	-0.14^{ns}	-0.28^{ns}	0.15^{ns}	-0.26^{ns}	**-0.50***	**0.45***	0.01^{ns}	-0.04^{ns}	-0.12^{ns}	0.16^{ns}
A	0.38^{ns}	0.05	1.00	-0.12^{ns}	**0.83****	-0.12^{ns}	**0.41***	-0.08^{ns}	0.08^{ns}	0.20^{ns}	0.09^{ns}	-0.19^{ns}	-0.05^{ns}	-0.05^{ns}	-0.33^{ns}	**-0.53***	0.04^{ns}	0.07^{ns}	-0.19^{ns}	0.27^{ns}	0.15^{ns}	0.22^{ns}	0.13^{ns}	0.18^{ns}
C_i	0.33^{ns}	0.10	0.29^{ns}	1.00	**-0.56***	**1.00****	-0.23^{ns}	-0.31^{ns}	-0.10^{ns}	0.07^{ns}	0.08^{ns}	0.22^{ns}	0.11^{ns}	0.02^{ns}	0.11^{ns}	0.11^{ns}	0.06^{ns}	0.03^{ns}	-0.31^{ns}	0.34^{ns}	0.13^{ns}	0.06^{ns}	0.01^{ns}	0.23^{ns}
A/C_i	-0.04^{ns}	-0.03	**0.45***	**-0.71****	1.00	-0.56*	**0.44***	0.05^{ns}	0.11^{ns}	0.14^{ns}	0.03^{ns}	-0.26^{ns}	-0.09^{ns}	-0.04^{ns}	-0.34^{ns}	**-0.51***	-0.03^{ns}	0.05^{ns}	0.01^{ns}	0.04^{ns}	0.06^{ns}	0.16^{ns}	0.10^{ns}	0.01^{ns}
C_i/C_a	0.33^{ns}	0.010	0.29^{ns}	**1.00****	-0.71^{ns}	1.00	-0.23^{ns}	-0.31^{ns}	-0.10^{ns}	0.07^{ns}	0.08^{ns}	0.22^{ns}	0.11^{ns}	0.02^{ns}	0.11^{ns}	0.11^{ns}	0.06^{ns}	0.03^{ns}	-0.31^{ns}	0.34^{ns}	0.13^{ns}	0.06^{ns}	0.01^{ns}	0.23^{ns}
WUE_i	**-0.54***	0.03	**0.56***	-0.02^{ns}	0.42^{ns}	-0.02^{ns}	1.00	0.06^{ns}	0.02^{ns}	-0.12^{ns}	-0.12^{ns}	-0.17^{ns}	-0.30^{ns}	-0.21^{ns}	-0.19^{ns}	-0.28^{ns}	0.13^{ns}	0.01^{ns}	-0.27^{ns}	0.33^{ns}	-0.07^{ns}	0.02^{ns}	0.02^{ns}	0.30^{ns}
WUE_{inst}	0.19^{ns}	**-0.80***	**0.54***	0.05^{ns}	0.32^{ns}	0.05^{ns}	0.32^{ns}	1.00	-0.32^{ns}	-0.28^{ns}	-0.08^{ns}	-0.19^{ns}	-0.16^{ns}	-0.19^{ns}	-0.04^{ns}	0.02^{ns}	-0.27^{ns}	0.29^{ns}	**0.42***	-0.35^{ns}	0.02^{ns}	0.16^{ns}	0.22^{ns}	-0.16^{ns}
F_o'	0.02^{ns}	0.07	0.01^{ns}	-0.03^{ns}	0.07^{ns}	-0.03^{ns}	-0.06^{ns}	-0.07^{ns}	1.00	**0.67***	**0.43***	0.12^{ns}	-0.22^{ns}	0.27^{ns}	0.20^{ns}	0.12^{ns}	0.17^{ns}	-0.11^{ns}	-0.17^{ns}	0.16^{ns}	-0.11^{ns}	-0.14^{ns}	-0.23^{ns}	-0.04^{ns}
F_m'	-0.03^{ns}	-0.09	0.26^{ns}	0.26^{ns}	-0.05^{ns}	0.26^{ns}	0.22^{ns}	0.21^{ns}	0.30^{ns}	1.00	**0.78****	0.02^{ns}	0.10^{ns}	**0.49***	-0.01^{ns}	-0.10^{ns}	0.03^{ns}	-0.03^{ns}	-0.06^{ns}	0.07^{ns}	0.16^{ns}	0.14^{ns}	0.06^{ns}	-0.12^{ns}
F_v/F_m	-0.09^{ns}	0.01	-0.11^{ns}	0.12^{ns}	-0.19^{ns}	0.12^{ns}	-0.07^{ns}	-0.08^{ns}	**0.56***	0.30^{ns}	1.00	0.02^{ns}	0.10^{ns}	**0.47***	-0.01^{ns}	-0.06^{ns}	-0.06^{ns}	0.25^{ns}	0.06^{ns}	0.02^{ns}	0.28^{ns}	0.18^{ns}	0.16^{ns}	-0.11^{ns}
Φ_{PSII}	-0.12^{ns}	-0.14	-0.35^{ns}	-0.12^{ns}	-0.16^{ns}	-0.12^{ns}	-0.20^{ns}	-0.08^{ns}	0.31^{ns}	0.10^{ns}	**0.42***	1.00	**0.46***	0.002^{ns}	**0.72****	**0.67***	0.19^{ns}	0.03^{ns}	0.16^{ns}	-0.17^{ns}	0.10^{ns}	-0.03^{ns}	-0.05^{ns}	-0.03^{ns}
qP	-0.26^{ns}	0.024	-0.23^{ns}	-0.26^{ns}	0.08^{ns}	-0.26^{ns}	0.04^{ns}	-0.13^{ns}	0.20^{ns}	0.14^{ns}	0.32^{ns}	0.42*	1.00	0.14^{ns}	0.22^{ns}	0.19^{ns}	0.22^{ns}	-0.01^{ns}	0.18^{ns}	-0.27^{ns}	0.05^{ns}	-0.11^{ns}	-0.02^{ns}	-0.01^{ns}
qN	-0.13^{ns}	-0.11	0.15^{ns}	0.05^{ns}	0.07^{ns}	0.05^{ns}	0.21^{ns}	0.15^{ns}	**0.57***	**0.66***	**0.72****	0.19^{ns}	0.07^{ns}	1.00	0.08^{ns}	0.10^{ns}	0.05^{ns}	-0.14^{ns}	-0.04^{ns}	-0.03^{ns}	0.24^{ns}	0.28^{ns}	0.35^{ns}	-0.07^{ns}
ETR	-0.15^{ns}	-0.08	-0.37^{ns}	-0.11^{ns}	-0.18^{ns}	-0.11^{ns}	-0.19^{ns}	-0.15^{ns}	0.32^{ns}	0.07^{ns}	**0.45***	**0.95****	0.41^{ns}	0.17^{ns}	1.00	**0.96****	-0.02^{ns}	-0.09^{ns}	0.06^{ns}	-0.12^{ns}	-001^{ns}	-0.13^{ns}	-0.10^{ns}	-0.07^{ns}
ETR/A	-0.28^{ns}	-0.07	**-0.67***	-0,20^{ns}	-0.30^{ns}	-0.20^{ns}	-0.35^{ns}	-0.33^{ns}	0.24^{ns}	-0.02^{ns}	0.43^{ns}	**0.88****	**0.44***	0.11^{ns}	0.93**	1.00	-0.04^{ns}	-0.11^{ns}	0.10^{ns}	-0.18^{ns}	-0.03^{ns}	-0.12^{ns}	-0.07^{ns}	-0.11^{ns}
AES	0.04^{ns}	0.02	0.03^{ns}	0.24^{ns}	-0.18^{ns}	0.24^{ns}	-0.07^{ns}	-0.04^{ns}	0.36^{ns}	0.25^{ns}	**0.51***	0.16^{ns}	-0.12^{ns}	**0.50***	0.14^{ns}	0.15^{ns}	1.00	-0.06^{ns}	-0.25^{ns}	0.27^{ns}	0.03^{ns}	-0.03^{ns}	-0.03^{ns}	0.11^{ns}
LA	-0.38^{ns}	-0.09	-0.27^{ns}	-0.02^{ns}	-0.17^{ns}	-0.02^{ns}	0.14^{ns}	-0.12^{ns}	-0.16^{ns}	0.01^{ns}	0.12^{ns}	0.08^{ns}	-0.01^{ns}	0.07^{ns}	0.13^{ns}	0.18^{ns}	0.05^{ns}	1.00	0.47*	-0.13^{ns}	0.18^{ns}	0.08^{ns}	0.11^{ns}	-0.13^{ns}
LDW	-0.39^{ns}	0.05	-0.09^{ns}	-0.12^{ns}	0.04^{ns}	-0.12^{ns}	0.29^{ns}	-0.07^{ns}	-0.05^{ns}	0.18^{ns}	0.17^{ns}	0.15^{ns}	-0.07^{ns}	0.35^{ns}	0.19^{ns}	0.18^{ns}	0.02^{ns}	**0.55****	1.00	**-0.93****	0.07^{ns}	0.03^{ns}	0.02^{ns}	**-0.56***
SLA	0.22^{ns}	-0.06	-0.07^{ns}	0.12^{ns}	-0.15^{ns}	0.12^{ns}	-0.26^{ns}	0.06^{ns}	-0.05^{ns}	-0.21^{ns}	-0.13^{ns}	-0.12^{ns}	0.10^{ns}	-0.38^{ns}	-0.14^{ns}	-0.10^{ns}	-0.01^{ns}	-0.05^{ns}	**-0.86****	1.00	-0.01^{ns}	0.01^{ns}	0.02^{ns}	**0.59***
TRL	0.02^{ns}	0.08	0.09^{ns}	0.19^{ns}	-0.14^{ns}	0.19^{ns}	0.11^{ns}	-0.04^{ns}	0.31^{ns}	0.31^{ns}	0.29^{ns}	0.26^{ns}	-0.11^{ns}	0.25^{ns}	0.29^{ns}	0.18^{ns}	0.12^{ns}	0.16^{ns}	0.08^{ns}	-0.01^{ns}	1.00	**0.84****	**0.75****	-0.10^{ns}
RDM	0.21^{ns}	-0.08	-0.03^{ns}	-0.04^{ns}	0.03^{ns}	-0.04^{ns}	-0.19^{ns}	0.05^{ns}	0.08^{ns}	0.14^{ns}	0.01^{ns}	0.22^{ns}	-0.20^{ns}	0.05^{ns}	0.25^{ns}	0.20^{ns}	0.12^{ns}	-0.05^{ns}	-0.05^{ns}	-0.03^{ns}	**0.46***	1.00	**0.88****	-0.12^{ns}
ERP	0.15^{ns}	0.02	0.15^{ns}	0.21^{ns}	-0.09^{ns}	0.21^{ns}	0.01^{ns}	0.05^{ns}	0.11^{ns}	0.02^{ns}	0.19^{ns}	**0.41***	-0.08^{ns}	0.07^{ns}	0.41*	0.25^{ns}	0.14^{ns}	-0.07^{ns}	-0.02^{ns}	-0.04^{ns}	**0.65***	**0.70****	1.00	-0.04^{ns}
Yield	0.27^{ns}	0.17	-0.03^{ns}	0.16^{ns}	-0.14^{ns}	0.16^{ns}	-0.25^{ns}	-0.16^{ns}	9E-4^{ns}	-0.25^{ns}	-0.08^{ns}	-0.17^{ns}	-0.13^{ns}	-0.27^{ns}	-0.18^{ns}	-0.14^{ns}	0.08^{ns}	0.04^{ns}	**-0.67***	**0.81****	0.11^{ns}	0.001^{ns}	0.02^{ns}	1.00

gs; stomatal conductance; ***T***, transpiration rate, ***A***; net CO_2 assimilation rate; ***A/C$_i$***, CO_2 assimilation rate/intercellular CO_2 concentration; ***C$_i$***, intercellular CO_2 concentration; ***C$_i$/C$_a$***, ratio of intercellular and atmospheric CO_2; ***WUE$_i$***, intrinsic water use efficiency; ***WUE$_{inst}$***, instantaneous water-use efficiency; ***F$_v$/F$_m$***, maximum quantum efficiency of photosystem II photochemistry; ***Φ$_{PSII}$***, the effective quantum efficiency of PSII photochemistry; ***qP***, photochemical quenching; ***qN***, non-photochemical quenching; ***ETR***, electron transport rate; ***ETR/A***, relative measure of electron transport to oxygen molecules; ***AES***, alternative electron sinks, ***LA***; leaf area, ***LDW***; leaf dry weight, ***SLA***; specific leaf area, ***TRL***; taproot length, ***RDM***; root dry mass, ***ERP***; estimated rooting percentage.* and ** denote significant at 5 and 1% probability levels, respectively. ns, non-significant

4.4 Discussion

Citron watermelon is a highly drought-tolerant crop and a useful source of drought-adaptive genes for breeding in cross-compatible and closely-related cucurbit species especially sweet watermelon. Previous studies assessed the drought tolerance of the crop based on seedling traits (Guzzon et al., 2017; Zhang et al., 2011b). Though these studies identified potential sources of drought tolerance for breeding, the magnitude of genotypic variation for drought tolerance in citron watermelon based on morphological and physiological traits is limiting for the efficient identification of potential sources for breeding and mapping of important genes associated with drought adaptation. Therefore, the present study assessed variation for drought tolerance among genetically diverse accessions of citron watermelon widely grown by small-holder farmers under water-restricted environments in the Limpopo Province of South Africa.

The study revealed varied responses for measured morphological and physiological traits among the assessed citron watermelon accessions. Water stress deficit impaired physiological processes such as reduced stomatal conductance, transpiration rate, and net CO_2 assimilation rate among the studied citron watermelon accessions (Table 4.4, Table 4.5 and Figure 4.2). Reduced stomatal conductance and transpiration rates are associated with water conservation enabling plants to avoid dehydration and loss of physiological functioning (Díaz-López et al., 2012; Ncama et al., 2022; Sousa et al., 2022). Reduced stomatal conductance, transpiration rates and photosynthetic activity observed for all accessions is indicative of efficient drought avoidance among the studied citron watermelon accessions. This allow the crop to survive under extreme drought conditions experienced in arid and semi-arid environments such as in the Limpopo Province where the accessions were collected.

The evaluated citron watermelon accessions exhibited high water-use efficiency under drought-stressed condition (Figure 4.2). These suggested the accessions are efficient water-users attributed to drought avoidance mechanisms such as reduced gas diffusion and transpiration rates (Table 4.4). Highly drought-tolerant accessions exhibited maximum WUE_i and WUE_{inst} compared to other accessions (Figure 4.2). Drought-tolerant genotypes use water more efficiently, maintain tissue water status, reduce water loss, and produce stable yield when water is limiting (Abreha et al., 2021; Mansour et al., 2021; Saad-Allah et al., 2021). The crop is able to produce high fruit yield with very limited rainfall. Of all cucurbit crops, farmers in most villages in the Limpopo Province prefer to plant citron watermelon due to its ability to produce high fodder and fruit yield under their water-limited environments which are used as animal feed during winter. Therefore, it is not surprising to observe the high levels of drought tolerance in this crop which are in agreement with farmer's claims about the high drought tolerance of the crop. In farmer's field, several accessions of the crop are planted resulting in cross-pollination and extensive phenotypic and genetic diversity reported in this crop (Mashilo et al., 2017c; Ngwepe et al., 2019) It is highly possible that genes and traits (e.g., morphological and physiological) conferring drought-adaptation including efficient water-loss regulation and efficient photosynthetic machinery are introgressed leading to extensive drought tolerance observed in the present study.

Citron watermelon is highly tolerant to oxidative stress induced by high light intensity (Mandizvo et al., 2021). This is associated with various photo-protection mechanisms that reduce damage to photosynthetic apparatus (Akashi et al., 2001b; Nanasato et al., 2005b; Takahara et al., 2005b; Yokota et al., 2002b; Yoshimura et al., 2008; Zhang et al., 2011a). In the present study, the highly tolerant citron watermelon accessions (*see* Figure 4.5) exhibited an efficient photosynthetic efficiency compared to sensitive accessions. An efficient PSII activity under drought stress condition prevents the formation of singlet oxygen, protecting the structure of chloroplasts from oxidative damage (Krieger-Liszkay et al., 2008; Pospisil and Prasad, 2014). Maintaining the efficiency of PSII function and the antioxidant system components are likely contributors to positive performance in tolerant accessions under drought stress condition. Furthermore, major energy dissipation processes non-photochemical quenching elevated under drought stress to enhance dissipation of excess energy in the photosynthetic apparatus (Figure 2n). In arid and semi-arid environments where citron watermelon is planted, drought and heat stress, and high light intensity are common and serious abiotic stress constraints. The long-term cultivation of the crop under these conditions led to the development of efficient photo-protection to prevent permanent damage of photosynthetic apparatus.

An increase in alternative electron sink (*AES*) was observed for all accessions under drought stress condition. Highly tolerant and tolerant accessions showed significantly higher *AES* values. In bottle gourd, Mashilo et al. (2017a) reported elevated *AES* is an indicator of drought stress tolerance. Increasing *AES* is a protective strategy to avoid excess energy at PSII reaction centres (Dambrosio et al., 2006; Zivcak et al., 2013). When an increase in *AES* accompanies CO_2 assimilation restriction, the water-water cycle (Cai et al., 2017) and photorespiration (Streb et al., 2005) dissipates excess excitation energy and balances the ATP/NADPH energy budget under drought stress condition. Further, Voss et al. (2013) reported the protection of photosynthesis from photoinhibition through *AES* (photorespiration) concurring with present findings.

Plants have evolved various drought-adaptive morphological responses to cope with drought stress under water-limiting conditions. Reduced plant growth and leaf area are important traits as they reduced water loss through transpiration. In the present study, the reduced leaf area among citron watermelon accessions is an indication of phenotypic flexibility and efficient drought-avoidance strategy. We observed reduced specific leaf area in the present study among all accessions. Specific leaf area is a useful measure of drought tolerance such that as drought decreases SLA, there is less possibility of water loss improving water-use efficiency (Queenborough and Porras, 2013).

Citron watermelon mean leaf area (Figure 4.4a) and specific leaf area (Figure 4.4c) were higher under non-stress condition. However, a shift in allometry (root to shoot metrics) were observed for taproot length (Figure 4.4d), root dry mass (Figure 4.4e) and estimated rooting percentage (Figure 4.4f) under drought stress condition. Citron watermelon root: shoot ratio changed in response to limiting resources above versus below ground. This change is supported by functional equilibrium theory, which suggests that plants shift allocation among absorptive tissue to acquire resources that limit growth (Lambers et al., 2008; Liu et al., 2022; Poorter and Nagel, 2000). The optimal partitioning theory takes also suggests that plants allocate resources among organs to optimize whole plant growth (Comas et al., 2013; Umaña et al., 2020; Weiner, 2004).

A well-developed root system architecture confers drought tolerance by acquiring water from a deep soil profile to support plant growth and development. Citron watermelon accessions had increased root dry mass, taproot length and estimated rooting percentage under drought stress condition (Table 4.10; Figure 4.4) compared to non-stressed condition. These indicated the ability of this crop to partition more dry matter to water acquiring parts (roots) than leaves when water is limiting (Figure 4.4). Present findings are in agreement with the theory of the functional balance proposed by Ritchie (1998), which predicts that plants will react to a limited water availability with a relative increase in the flow of assimilates to the root leading to an increased root dry mass and volume.

In most villages where the crop is cultivated, it is often planted in poor sandy soils with poor water retention capacity. This led to the crop developing a deep root system for water acquisition. Recently, root system excavation of various citron watermelon accessions planted under field conditions exposed a thick tap-root system extending to about 10 cm long with three to seven lateral roots extending over 2 m long (J. Mashilo, personal observation). In agreement with these observations, (Katuuramu et al., 2020) reported total root length varying from 103.32 to 171.58 cm among citron watermelon accessions.

This was higher compared to values ranging from 8.78 to 137.20 cm reported for sweet watermelon (Katuuramu et al., 2020). Other root traits including total root surface area, average root diameter and total root volume were reportedly higher in citron watermelon than sweet watermelon (Katuuramu et al., 2020). These observations agree with present findings, indicating that citron watermelon develops a deep root system architecture to allow deep water access to produce high biomass and fruit yield under water-constrained environments. As evidenced by visual aid (Figure 4.4), drought stress induced a deep root system as a conservative balance between water-losing organs (leaves) and water obtaining organs (roots) in the evaluated citron watermelon accessions. Some attributes including the deep root system of citron watermelon has made it the “new” preferred rootstock for improving fruit and quality of grafted sweet watermelon for dry water-limited environments (Seymen et al., 2021; Thies et al., 2015a; Thies et al., 2015b; Thies et al., 2015c; Yavuz et al., 2020).

Understanding the interrelationships among agronomic, physiological, and morphological traits can provide useful information for an integrated drought tolerance breeding approach in breeding programs. The positive associations observed between fruit yield and various physiological and morphological traits under drought stress condition including WUE_{inst} (r = 0.97), LDW (r = 0.77), TRL (r =0.46) and RDM (r = 0.48) suggested simultaneous selection and improvement of these traits in citron watermelon (Figure 4.10).

4.5 Conclusion

The citron watermelon accessions evaluated in the present study showed varying levels of drought tolerance based on morphological and physiological traits. These allowed five distinct groupings namely: A (highly drought-tolerant), B (drought-tolerant), C (moderately drought-tolerant), D (drought-sensitive) and E (highly drought-sensitive) based on various drought tolerance indices. The following accessions (WWM-02, WWM-05, WWM-09, WWM-15, WWM-37(2), WWM-39, WWM-41(A), WWM-46, WWM-47, WWM-57, WWM-64, WWM-66, WWM-68 and WWM-79) were categorized as highly-drought tolerant and accessions WWM-03, WWM-08, WWM-14, WWM-21, WWM-33, WWM-35(1), WWM-35(2), WWM-67 and WWM-76 as drought tolerant. These are useful genetic stock for genetic improvement of drought tolerance in this crop and related cucurbit crop including sweet watermelon.

References

Abreha K.B., Enyew M., Carlsson A.S., Vetukuri R.R., Feyissa T., Motlhaodi T., Ng'uni D., Geleta M. (2021) Sorghum in dryland: morphological, physiological, and molecular responses of sorghum under drought stress. Planta 255:20. DOI: 10.1007/s00425-021-03799-7.

Akashi K., Miyake C., Yokota A. (2001a) Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. FEBS Letters 508:438-442. DOI: 10.1016/s0014-5793(01)03123-4.

Akashi K., Miyake C., Yokota A. (2001b) Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. FEBS Letters 508:438-442.

Akashi K., Takahara K., Yamada R., Miyake C., Yokota A. (2002) Functional analysis of DRIP-1, a drought-induced polypeptide in wild watermelon. Plant and Cell Physiology 43:S112-S112.

Arifuzzaman M., Oladzadabbasabadi A., McClean P., Rahman M. (2019) Shovelomics for phenotyping root architectural traits of rapeseed/canola (*Brassica napus* L.) and genome-wide association mapping. Mol Genet Genomics 294:985-1000. DOI: 10.1007/s00438-019-01563-x.

Ayed S., Othmani A., Bouhaouel I., Teixeira da Silva J.A. (2021) Multi-Environment Screening of Durum Wheat Genotypes for Drought Tolerance in Changing Climatic Events. Agronomy 11. DOI: 10.3390/agronomy11050875.

Bhusal N., Lee M., Lee H., Adhikari A., Han A.R., Han A., Kim H.S. (2021) Evaluation of morphological, physiological, and biochemical traits for assessing drought resistance in eleven tree species. Sci Total Environ 779:146466. DOI: 10.1016/j.scitotenv.2021.146466.

Bikdeloo M., Colla G., Rouphael Y., Hassandokht M.R., Soltani F., Salehi R., Kumar P., Cardarelli M. (2021) Morphological and Physio-Biochemical Responses of Watermelon Grafted onto Rootstocks of Wild Watermelon [*Citrullus colocynthis* (L.) Schrad] and Commercial Interspecific Cucurbita Hybrid to Drought Stress. Horticulturae 7. DOI: 10.3390/horticulturae7100359.

Boyer J.S. (1982) Plant productivity and environment. Science 218:443-8. DOI: 10.1126/science.218.4571.443.

Bucksch A., Burridge J., York L.M., Das A., Nord E., Weitz J.S., Lynch J.P. (2014) Image-based high-throughput field phenotyping of crop roots. Plant Physiol 166:470-86. DOI: 10.1104/pp.114.243519.

Cai Y.F., Yang Q.Y., Li S.F., Wang J.H., Huang W. (2017) The water-water cycle is a major electron sink in *Camellia* species when CO_2 assimilation is restricted. J Photochem Photobiol B 168:59-66. DOI: 10.1016/j.jphotobiol.2017.01.024.

Comas L.H., Becker S.R., Cruz V.M., Byrne P.F., Dierig D.A. (2013) Root traits contributing to plant productivity under drought. Front Plant Sci 4:442. DOI: 10.3389/fpls.2013.00442.

Dambrosio N., Arena C., Desanto A. (2006) Temperature response of photosynthesis, excitation energy dissipation and alternative electron sinks to carbon assimilation in *Beta vulgaris* L. Environmental and Experimental Botany 55:248-257. DOI: 10.1016/j.envexpbot.2004.11.006.

Díaz-López L., Gimeno V., Simón I., Martínez V., Rodríguez-Ortega W.M., García-Sánchez F. (2012) *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. Agricultural Water Management 105:48-56. DOI: 10.1016/j.agwat.2012.01.001.

Easlon H.M., Bloom A.J. (2014) Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. Appl Plant Sci 2. DOI: 10.3732/apps.1400033.

Edelstein M., Tyutyunik J., Fallik E., Meir A., Tadmor Y., Cohen R. (2014) Horticultural evaluation of exotic watermelon germplasm as potential rootstocks. Scientia Horticulturae 165:196-202. DOI: 10.1016/j.scienta.2013.11.010.

Evans J.R. (2009) Potential errors in electron transport rates calculated from chlorophyll fluorescence as revealed by a multilayer leaf model. Plant Cell Physiol 50:698-706. DOI: 10.1093/pcp/pcp041.

Fernandez G.C.J. (1992) Effective selection criteria for assessing plant stress tolerance. Proceeding of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, Aug. 13-16, Shanhua, Taiwan, 1992:257-270.

Fischer R.A., Maurer R. (1978) Drought resistance in spring wheat cultivars. I. Grain yield responses. Australian Journal of Agricultural Research 29. DOI: 10.1071/ar9780897.

García-Mendívil H.A., Munera M., Giné A., Escudero N., Picó M.B., Gisbert C., Sorribas F.J. (2019) Response of two *Citrullus amarus* accessions to isolates of three species of *Meloidogyne* and their graft compatibility with watermelon. Crop Protection 119:208-213. DOI: 10.1016/j.cropro.2019.02.005.

Gavuzzi P., Rizza F., Palumbo M., Campanile R.G., Ricciardi G.L., Borghi B. (1997) Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. Canadian Journal of Plant Science 77:523-531. DOI: 10.4141/p96-130.

Grzesiak S., Hordyńska N., Szczyrek P., Grzesiak M.T., Noga A., Szechyńska-Hebda M. (2018) Variation among wheat (*Triticum easativum* L.) genotypes in response to the drought stress: I – selection approaches. Journal of Plant Interactions 14:30-44. DOI: 10.1080/17429145.2018.1550817.

Guzzon F., Müller J.V., do Nascimento Araujo M., Cauzzi P., Orsenigo S., Mondoni A., Abeli T. (2017) Drought avoidance adaptive traits in seed germination and seedling growth of Citrullus amarus landraces. South African Journal of Botany 113:382-388. DOI: 10.1016/j.sajb.2017.09.023.

Hakki E.E., Hamurcu M., Gezgin S., Demiral T. (2016) Physiological responses of two contrasting watermelon genotypes exposed to drought and nitric oxide. Journal of Biotechnology 231:S25-S25. DOI: 10.1016/j.jbiotec.2016.05.110.

Kajikawa M., Morikawa K., Abe Y., Yokota A., Akashi K. (2010) Establishment of a transgenic hairy root system in wild and domesticated watermelon (*Citrullus lanatus*) for studying root vigor under drought. Plant Cell Rep 29:771-8. DOI: 10.1007/s00299-010-0863-3.

Kashyap B., Kumar R. (2021) Sensing Methodologies in Agriculture for Soil Moisture and Nutrient Monitoring. IEEE Access 9:14095-14121. DOI: 10.1109/access.2021.3052478.

Katuuramu D.N., Wechter W.P., Washington M.L., Horry M., Cutulle M.A., Jarret R.L., Levi A. (2020) Phenotypic diversity for root traits and identification of superior germplasm for root breeding in watermelon. HortScience 55:1272-1279. DOI: 10.21273/hortsci15093-20.

Kitao M., Lei T.T., Koike T., Tobita H., Maruyama Y. (2003) Higher electron transport rate observed at low intercellular CO_2 concentration in long-term drought-acclimated leaves of Japanese mountain birch (*Betula ermanii*). Physiologia Plantarum 118:406-413. DOI: 10.1034/j.1399-3054.2003.00120.x.

Kombo M.D., Sari N. (2019) Rootstock effects on seed yield and quality in watermelon. Horticulture, Environment, and Biotechnology 60:303-312. DOI: 10.1007/s13580-019-00131-x.

Krieger-Liszkay A., Fufezan C., Trebst A. (2008) Singlet oxygen production in photosystem II and related protection mechanism. Photosynth Res 98:551-64. DOI: 10.1007/s11120-008-9349-3.

Lambers H., Chapin F.S., Pons T.L. (2008) Plant Physiological Ecology Springer Science & Business Media.

Liu A.M., Latimer J.G. (1995) Water Relations and Abscisic-Acid Levels of Watermelon as Affected by Rooting Volume Restriction. Journal of Experimental Botany 46:1011-1015. DOI: DOI 10.1093/jxb/46.8.1011.

Liu H., Wang H., Li N., Shao J., Zhou X., van Groenigen K.J., Thakur M.P. (2022) Phenological mismatches between above- and belowground plant responses to climate warming. Nature Climate Change 12:97-102. DOI: 10.1038/s41558-021-01244-x.

Malambane G., Batlang U., Ramolekwa K., Tsujimoto H., Akashi K. (2021) Growth chamber and field evaluation of physiological factors of two watermelon genotypes. Plant Stress 2. DOI: 10.1016/j.stress.2021.100017.

Malambane G., Nonaka S., Shiba H., Ezura H., Tsujimoto H., Akashi K. (2018) Comparative effects of ethylene inhibitors on Agrobacterium-mediated transformation of drought-tolerant wild watermelon. Biosci Biotechnol Biochem 82:433-441. DOI: 10.1080/09168451.2018.1431516.

Mandizvo T., Odindo A.O., Mashilo J. (2021) Citron Watermelon Potential to Improve Crop Diversification and Reduce Negative Impacts of Climate Change. Sustainability 13. DOI: 10.3390/su13042269.

Mandizvo T., Odindo A.O., Mashilo J. (2022) Nutrient composition and physical properties of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) seeds are related to seed coat visual appearance. South African Journal of Botany. DOI: 10.1016/j.sajb.2022.02.015.

Mansour E., Desoky E.-S.M., Ali M.M.A., Abdul-Hamid M.I., Ullah H., Attia A., Datta A. (2021) Identifying drought-tolerant genotypes of faba bean and their agro-physiological responses to different water regimes in an arid Mediterranean environment. Agricultural Water Management 247. DOI: 10.1016/j.agwat.2021.106754.

Mashilo J., Odindo A., Shimelis H., Musenge P.A., Tesfay S.Z., Magwaza L.S. (2018a) Photosynthetic efficiency of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] under drought stress. Indian Journal of Plant Physiology 23:293-304. DOI: 10.1007/s40502-018-0377-5.

Mashilo J., Odindo A.O., Shimelis H.A., Musenge P., Tesfay S.Z., Magwaza L.S. (2017a) Drought tolerance of selected bottle gourd [*Lagenaria siceraria* (Molina) Standl.] landraces assessed by leaf gas exchange and photosynthetic efficiency. Plant Physiol Biochem 120:75-87. DOI: 10.1016/j.plaphy.2017.09.022.

Mashilo J., Odindo A.O., Shimelis H.A., Musenge P., Tesfay S.Z., Magwaza L.S. (2018b) Photosynthetic response of bottle gourd [*Lagenaria siceraria* (Molina) Standl.] to drought stress: Relationship between cucurbitacins accumulation and drought tolerance. Scientia Horticulturae 231:133-143. DOI: 10.1016/j.scienta.2017.12.027.

Mashilo J., Shimelis H., Ngwepe R.M. (2021) Genetic resources of bottle gourd (*Lagenaria siceraria* (Molina) Standl.] and citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.): implications for genetic improvement, product development and commercialization: a review. South African Journal of Botany. DOI: 10.1016/j.sajb.2021.10.013.

Mashilo J., Shimelis H., Odindo A.O., Amelework B. (2017b) Genetic diversity and differentiation in citron watermelon [Citrullus lanatus var. citroides] landraces assessed by simple sequence repeat markers. Scientia Horticulturae 214:99-106. DOI: 10.1016/j.scienta.2016.11.015.

Mashilo J., Shimelis H., Odindo A.O., Amelework B. (2017c) Genetic diversity and differentiation in citron watermelon [*Citrullus lanatus* var. *citroides*] landraces assessed by simple sequence repeat markers. Scientia Horticulturae 214:99-106. DOI: 10.1016/j.scienta.2016.11.015.

Medrano H., Tomás M., Martorell S., Flexas J., Hernández E., Rosselló J., Pou A., Escalona J.-M., Bota J. (2015) From leaf to whole-plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as a selection target. The Crop Journal 3:220-228. DOI: 10.1016/j.cj.2015.04.002.

Minsart L.A., Zoro bi I.A., Djè Y., Baudoin J.P., Jacquemart A.L., Bertin P. (2010) Set up of simple sequence repeat markers and first investigation of the genetic diversity of West-African watermelon (*Citrullus lanatus* ssp. vulgaris oleaginous type). Genetic Resources and Crop Evolution 58:805-814. DOI: 10.1007/s10722-010-9617-x.

Mo Y., Yang R., Liu L., Gu X., Yang X., Wang Y., Zhang X., Li H. (2015) Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. Plant Growth Regulation 79:229-241. DOI: 10.1007/s10725-015-0128-9.

Mo Y., Yang R., Liu L., Gu X., Yang X., Wang Y., Zhang X., Li H. (2016) Growth, photosynthesis and adaptive responses of wild and domesticated watermelon genotypes to drought stress and subsequent re-watering. Plant Growth Regulation 79:229-241. DOI: 10.1007/s10725-015-0128-9.

Nanasato Y., Akashi K., Yokota A. (2005a) Co-expression of cytochrome b_{561} and ascorbate oxidase in leaves of wild watermelon under drought and high light conditions. Plant Cell Physiol 46:1515-24. DOI: 10.1093/pcp/pci164.

Nanasato Y., Akashi K., Yokota A. (2005b) Co-expression of cytochrome b_{561} and ascorbate oxidase in leaves of wild watermelon under drought and high light conditions. Plant and Cell Physiology 46:1515-1524.

Ncama K., Aremu O.A., Sithole N.J. (2022) Plant Adaptation to Environmental Stress: Drought, Chilling, Heat, and Salinity, Environment and Climate-smart Food Production, Springer. pp. 151-179.

Ngwepe R.M., Mashilo J., Shimelis H. (2019) Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides*): a review. Genetic Resources and Crop Evolution 66:735-758. DOI: 10.1007/s10722-018-0724-4.

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

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

Nkoana D.K., Mashilo J., Shimelis H., Ngwepe R.M. (2021) Nutritional, phytochemical compositions and natural therapeutic values of citron watermelon (*Citrullus lanatus* var. *citroides*): A Review. South African Journal of Botany. DOI: 10.1016/j.sajb.2020.12.008.

Ort D.R., Baker N.R. (2002) A photoprotective role for O_2 as an alternative electron sink in photosynthesis? Current Opinion in Plant Biology 5:193-198. DOI: 10.1016/s1369-5266(02)00259-5.

Pal S., Rao E.S., Hebbar S.S., Sriram S., Pitchaimuthu M., Keshava Rao V. (2020) Assessment of Fusarium wilt resistant *Citrullus* sp. rootstocks for yield and quality traits of grafted watermelon. Scientia Horticulturae 272. DOI: 10.1016/j.scienta.2020.109497.

Patel J.M., Patel A.S., Patel C.R., Mamrutha H.M., Pradeep S., Pachchigar K.P. (2019) Evaluation of Selection Indices in Screening Durum Wheat Genotypes Combining Drought Tolerance and High Yield Potential. International Journal of Current Microbiology and Applied Sciences 8:1165-1178. DOI: 10.20546/ijcmas.2019.804.134.

Poorter H., Nagel O. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Functional Plant Biology 27. DOI: 10.1071/pp99173_co.

Pospisil P., Prasad A. (2014) Formation of singlet oxygen and protection against its oxidative damage in Photosystem II under abiotic stress. J Photochem Photobiol B 137:39-48. DOI: 10.1016/j.jphotobiol.2014.04.025.

Qin J., Bian C., Liu J., Zhang J., Jin L. (2019) An efficient greenhouse method to screen potato genotypes for drought tolerance. Scientia Horticulturae 253:61-69. DOI: 10.1016/j.scienta.2019.04.017.

Queenborough S.A., Porras C. (2013) Expanding the coverage of plant trait databases – A comparison of specific leaf area derived from fresh and dried leaves. Plant Ecology & Diversity 7:383-388. DOI: 10.1080/17550874.2013.777483.

Ritchie J.T. (1998) Soil water balance and plant water stress, Understanding options for agricultural production, Springer, Dordrecht, Netherlands, Dordrecht. pp. 41-54.

Rosielle A.A., Hamblin J. (1981) Theoretical aspects of selection for yield in stress and non-stress environment. Crop Science 21:943-946. DOI: 10.2135/cropsci1981.0011183X002100060033x.

Saad-Allah K.M., Nessem A.A., Ebrahim M.K.H., Gad D. (2021) Evaluation of Drought Tolerance of Five Maize Genotypes by Virtue of Physiological and Molecular Responses. Agronomy 12. DOI: 10.3390/agronomy12010059.

Sánchez-Virosta Á., Sadras V.O., Sánchez-Gómez D. (2021) Phenotypic plasticity in relation to inter-cultivar variation of garlic (*Allium sativum* L.) functional performance and yield-stability in response to water availability. Scientia Horticulturae 285. DOI: 10.1016/j.scienta.2021.110128.

Schneider K.A., Rosales-Serna R., Ibarra-Perez F., Cazares-Enriquez B., Acosta-Gallegos J.A., Ramirez-Vallejo P., Wassimi N., Kelly J.D. (1997) Improving Common Bean Performance under Drought Stress. Crop Science 37:43-50. DOI: 10.2135/cropsci1997.0011183X003700010007x.

Seymen M., Yavuz D., Ercan M., Akbulut M., Çoklar H., Kurtar E.S., Yavuz N., Süheri S., Türkmen Ö. (2021) Effect of wild watermelon rootstocks and water stress on chemical properties of watermelon fruit. Horticulture, Environment, and Biotechnology 62:411-422. DOI: 10.1007/s13580-020-00329-4.

Singh D., Singh R., Sandhu J.S., Chunneja P. (2017) Morphological and genetic diversity analysis of Citrullus landraces from India and their genetic inter relationship with continental watermelons. Scientia Horticulturae 218:240-248. DOI: 10.1016/j.scienta.2017.02.013.

Solmaz I., Sari N., Aka-Kacar Y., Yalcin-Mendi N.Y. (2010) The genetic characterization of Turkish watermelon (*Citrullus lanatus*) accessions using RAPD markers. Genetic Resources and Crop Evolution 57:763-771. DOI: 10.1007/s10722-009-9515-2.

Song Q., Joshi M., DiPiazza J., Joshi V. (2020) Functional Relevance of Citrulline in the Vegetative Tissues of Watermelon During Abiotic Stresses. Front Plant Sci 11:512. DOI: 10.3389/fpls.2020.00512.

Sousa A.R.d.O., Silva E.M.d.A., Filho M.A.C., Costa M.G.C., Filho W.d.S.S., Micheli F., Maserti B., Gesteira A.d.S. (2022) Metabolic responses to drought stress and rehydration in leaves and roots of three Citrus scion/rootstock combinations. Scientia Horticulturae 292. DOI: 10.1016/j.scienta.2021.110490.

Stone S., Boyhan G., McGregor C. (2019) Inter- and Intracultivar Variation of Heirloom and Open-pollinated Watermelon Cultivars. HortScience 54:212-220. DOI: 10.21273/hortsci13638-18.

Streb P., Josse E.-M., Gallouet E., Baptist F., Kuntz M., Cornic G. (2005) Evidence for alternative electron sinks to photosynthetic carbon assimilation in the high mountain plant species Ranunculus glacialis. Plant, Cell and Environment 28:1123-1135. DOI: 10.1111/j.1365-3040.2005.01350.x.

Takahara K., Akashi K., Yokota A. (2005a) Biochemical and molecular characterization of glutamate N-acetyltransferase involved in citrulline accumulation in wild watermelon during drought/strong-light stresses. Plant and Cell Physiology 46:S74-S74.

Takahara K., Akashi K., Yokota A. (2005b) Purification and characterization of glutamate N-acetyltransferase involved in citrulline accumulation in wild watermelon. FEBS Journal 272:5353-5364.

Takahara K., Yoshino A., Akashi K., Yokota A. (2006) Regulation of metabolic pathways for the massive accumulation of citrulline during drought/strong light stress in wild watermelon. Plant and Cell Physiology 47:S102-S102.

Thies J.A., Ariss J.J., Hassell R.L., Buckner S., Levi A. (2015a) Accessions of *Citrullus lanatus* var. *citroide*s are valuable rootstocks for grafted watermelon in fields infested with root-knot nematodes. HortScience 50:4-8. DOI: 10.21273/hortsci.50.1.4.

Thies J.A., Buckner S., Horry M., Hassell R., Levi A. (2015b) Influence of *Citrullus lanatus* var. *citroides* rootstocks and their F1 hybrids on yield and response to root-knot nematode, *Meloidogyne incognita*, in grafted watermelon. HortScience 50:9-12. DOI: 10.21273/hortsci.50.1.9.

Thies J.A., Levi A., Ariss J.J., Hassell R.L. (2015c) RKVL-318, a root-knot nematode-resistant watermelon line as rootstock for grafted watermelon. HortScience 50:141-142. DOI: 10.21273/hortsci.50.1.141.

Umaña M.N., Cao M., Lin L., Swenson N.G., Zhang C., Liu X. (2020) Trade-offs in above- and below-ground biomass allocation influencing seedling growth in a tropical forest. Journal of Ecology 109:1184-1193. DOI: 10.1111/1365-2745.13543.

Voss I., Sunil B., Scheibe R., Raghavendra A.S. (2013) Emerging concept for the role of photorespiration as an important part of abiotic stress response. Plant Biol (Stuttg) 15:713-22. DOI: 10.1111/j.1438-8677.2012.00710.x.

Webster R.J., Driever S.M., Kromdijk J., McGrath J., Leakey A.D., Siebke K., Demetriades-Shah T., Bonnage S., Peloe T., Lawson T. (2016) High C_3 photosynthetic capacity and high intrinsic water use efficiency underlies the high productivity of the bioenergy grass *Arundo donax*. Scientific reports 6:1-10.

Weiner J. (2004) Allocation, plasticity and allometry in plants. Perspectives in Plant Ecology, Evolution and Systematics 6:207-215. DOI: 10.1078/1433-8319-00083.

Yang X., Ren R., Ray R., Xu J., Li P., Zhang M., Liu G., Yao X., Kilian A. (2016) Genetic diversity and population structure of core watermelon (*Citrullus lanatus*) genotypes using DArTseq-based SNPs. Plant Genetic Resources 14:226-233. DOI: 10.1017/s1479262115000659.

Yavuz D., Seymen M., Süheri S., Yavuz N., Türkmen Ö., Kurtar E.S. (2020) How do rootstocks of citron watermelon (*Citrullus lanatus* var. *citroides*) affect the yield and quality of watermelon under deficit irrigation? Agricultural Water Management 241. DOI: 10.1016/j.agwat.2020.106351.

Yokota A., Kawasaki S., Iwano M., Nakamura C., Miyake C., Akashi K. (2002a) Citrulline and DRIP-1 protein (ArgE homologue) in drought tolerance of wild watermelon. Ann Bot 89 Spec No:825-32. DOI: 10.1093/aob/mcf074.

Yokota A., Kawasaki S., Iwano M., Nakamura C., Miyake C., Akashi K. (2002b) Citrulline and DRIP-1 protein (ArgE homologue) in drought tolerance of wild watermelon. Annals of Botany 89:825-832.

Yoshimura K., Masuda A., Kuwano M., Yokota A., Akashi K. (2008) Programmed proteome response for drought avoidance/tolerance in the root of a C3 xerophyte (wild watermelon) under water deficits. Plant and Cell Physiology 49:226-241. DOI: 10.1093/pcp/pcm180.

Zhang H., Fan J., Guo S., Ren Y., Gong G., Zhang J., Weng Y., Davis A., Xu Y. (2016) Genetic Diversity, Population Structure, and Formation of a Core Collection of 1197 Citrullus Accessions. HortScience 51:23-29. DOI: 10.21273/hortsci.51.1.23.

Zhang H., Gong G., Guo S., Ren Y., Xu Y., Ling K.S. (2011a) Screening the USDA watermelon germplasm collection for drought tolerance at the seedling stage. HortScience 46:1245-1248.

Zhang H.Y., Gong G.Y., Guo S.G., Ren Y., Xu Y., Ling K.S. (2011b) Screening the USDA Watermelon Germplasm Collection for Drought Tolerance at the Seedling Stage. Hortscience 46:1245-1248. DOI: 10.21273/hortsci.46.9.1245.

Zivcak M., Brestic M., Balatova Z., Drevenakova P., Olsovska K., Kalaji H.M., Yang X., Allakhverdiev S.I. (2013) Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. Photosynth Res 117:529-46. DOI: 10.1007/s11120-013-9885-3.

Zlatev Z. (2014) Drought-Induced Changes in Chlorophyll Fluorescence of Young Wheat Plants. Biotechnology & Biotechnological Equipment 23:438-441. DOI: 10.1080/13102818.2009.10818458.

Chapter 5: Phenotypic variability of root system architecture traits for drought tolerance among accessions of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey)

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Abstract

Citron watermelon (*Citrullus lanatus* var. *citroides*) is a drought-tolerant cucurbit crop widely grown in sub-Saharan Africa in arid and semi-arid environments. The species is a C3 xerophyte used for multiple purposes, including intercropping with maize and has a deep taproot system. The deep taproot system plays a key role in the species' adaptation to dry conditions. Understanding root system development of this crop could be useful to identify traits for breeding water-use efficient and drought-tolerant varieties. This study compared root system architecture of citron watermelon accessions under water-stress conditions. Nine selected and drought-tolerant citron watermelon accessions were grown under non-stress (NS) and water stress (WS) conditions using the root rhizotron procedure in a glasshouse. The following root system architecture (RSA) traits were measured, namely: root system width (RSW), root system depth (RSD), convex hull area (CHA), total root length (TRL), root branch count (RBC), total root volume (TRV), leaf area (LA), leaf number (LN), first seminal root length (FSRL), seminal root angle (SRA), root dry mass (RDM), shoot dry mass (SDM), root–shoot mass ratio (RSM), root mass ratio (RMR), shoot mass ratio (SMR) and root tissue density (RTD). The data collected on RSA traits were subjected to the analysis of variance (ANOVA), correlation and principal component analyses. ANOVA revealed a significant ($p < 0.05$) accession × water stress interaction effect for studied RSA traits. Under WS, RDM exhibited significant and positive correlations with RSM ($r = 0.65$), RMR ($r = 0.66$), RSD ($r = 0.66$), TRL ($r = 0.60$), RBC ($r = 0.72$), FSRL ($r = 0.73$) and LN ($r = 0.70$). Principal component analysis revealed high loading scores for the following RSA traits: RSW (0.89), RSD (0.97), TRL (0.99), TRV (0.90), TRL (0.99), RMR (0.96) and RDM (0.76). In conclusion, the study has shown that the identified RSA traits could be useful in crop improvement programmes for citron watermelon genotypes with enhanced drought adaptation for improved yield performance under drought-prone environments.

Keywords: Biomass partitioning; Digital root phenotyping; Image analysis; Rhizotron; Root architecture; Root phenes; RootSnap

5.1 Introduction

Citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) is an important cucurbit crop grown for multiple purposes as human food and animal feed in many parts of Africa. For use as food, several plant parts of the crop are consumed, namely: fresh leaves, ripened fruit and seed which provide essential nutrients and phytochemical compounds (Mandizvo et al., 2022a; Mashilo et al., 2021; Nkoana et al., 2021). The fresh or dried vines are used as feed for domesticated animals (Mandizvo et al., 2021; Mashilo et al., 2021). Of the cucurbit crops, citron watermelon is the most drought and heat tolerant (Mandizvo et al., 2021). Also, the crop is efficient to absorb N (Hong et al., 2022; Yavuz et al., 2020); and is tolerant to biotic stresses including pathogenic diseases such as *Fusarium* wilt, gummy stem blight, bacterial fruit blotch, powdery mildew, viral diseases and root-knot nematodes (Katuuramu et al., 2021; Maja et al., 2021; Ngwepe et al., 2019). Due to these desirable attributes, citron watermelon is presently being explored as a suitable rootstock for improving biotic and abiotic stress tolerance and fruit quality of grafted sweet watermelon (Katuuramu et al., 2020; Schwarz et al., 2010). In addition, citron watermelon is a preferred cucurbit crop for gene introgression and breeding in sweet watermelon.

Citron watermelon has a deep and well-developed root system (high root branch count, root length and convex hull area) than most cucurbit crops including sweet watermelon (*Citrullus lanatus* subsp. *vulgaris* Achigan-Dako 06NIA 224 (GAT) Benin), tendril-less melon (*Citrullus ecirrhosus* Griffin 16056 (M) Namibia), and Egusi watermelon (*Citrullus mucosospermus* Vavilov CIT 204 (GAT) Benin) (Chomicki and Renner, 2015; Katuuramu et al., 2020). The well-developed root system may explain the species ability to tolerate drought conditions and produce optimum fruit yield under drought-prone environments. Therefore, understanding root morphology development in this species under water-restricted environments will add useful information for improved yield performance. The root system architecture (RSA) has a high degree of plasticity, allowing the plant to acclimate changing environmental conditions (Gloaguen et al., 2022; Koevoets et al., 2016). Plant plasticity is important to competitiveness and resilience to changing environmental conditions (de Kroon et al., 2005; Koevoets et al., 2016). Soil moisture is an important environmental factor that impacts the RSA traits. For example, sweet watermelon has been reported to have enhanced root development under low soil moisture conditions (Uygur and Yetisir, 2009). In citron watermelon, little information is available regarding RSA development and how soil moisture impacts the development of below-ground plant organs.

The citron watermelon root architecture has a primary taproot and several lateral roots (Yavuz et al., 2020). Genetic variability has been reported in the species for morphological traits including RSA traits (Crosby et al., 2008; Fita et al., 2007; Mashilo et al., 2016; Mashilo et al., 2017a; Mashilo et al., 2017b; Omirou et al., 2013; Rentería-Martínez et al., 2018; Roy et al., 2012; Thies and Levi, 2007). Using a genetically diverse population of citron watermelon accessions collected and grown in the drier parts of South Africa by small-holder farmers, Mandizvo et al. (2022b) observed variability in estimated root percentage, taproot length and root dry mass among the accessions after exposure to drought stress. The authors observed that some accessions maintained either a lower or higher root biomass independent of growth conditions (e.g., non-stressed or water-stressed), suggesting a substantial genetic control of RSA traits in citron watermelon.

The observed phenotypic variability in citron watermelon RSA traits suggests that there could be underlying genetic variation among citron watermelon landraces in relation to root morphology development and architecture under drought conditions. Therefore, understanding root system development under drought conditions in this species could aid in breeding high-yielding and improved cultivars with enhanced water-use and drought-tolerance traits adapted to dry conditions, which are further exacerbated by prolonged dry spells and erratic rainfall as a result of climate change. The objective of this work was to study root system architecture of citron watermelon accessions and identify drought-adaptive root traits for cultivar improvement under water-stressed environments.

5.2 Materials and methods

5.2.1 Plant material

The Department of Agriculture and Rural Development (DARD), Bela-Bela, Limpopo Province, South Africa, provided citron watermelon accessions for the study. Out of forty citron watermelon accessions, nine accessions classified as "*highly drought-tolerant*" by Agglomerative Hierarchical Clustering (AHC) using six drought indices from our previous study (Mandizvo et al., 2022b) were used for root phenotyping. Based on our previous study findings Mandizvo et al. (2022b), each accession's drought stress tolerance index is summarised in Table 5.1.

Table 5.1: Information on the source of seed, drought stress tolerance index and seed coat colour of citron watermelon accessions used in the study

Accession	District	Village	Latitude and Longitude	STI	Seed coat colour
WWM-09	Capricorn	Moletjie-Moshate	23°36'55.9"S 29°16'03.7"E	0.452	nut brown
WWM-15	Capricorn	Turfloop	23°53'12.2"S 29°44'52.2"E	0.417	tomato red
WWM-37(2)	Capricorn	Ga-Molepo	24°01'11.1"S 29°47'05.0"E	0.392	purple violet
WWM-39	Capricorn	Ga-Mphela	23°43'19.2"S 29°12'01.4"E	0.431	ruby red
WWM-41(A)	Sekhukhune	Nebo	24°54'09.1"S 29°46'15.8"E	0.434	purple red
WWM-46	Sekhukhune	Nebo	24°54'07.2"S 29°46'13.2"E	0.459	signal red
WWM-64	Capricorn	Ga-Mphela	23°39'46.0"S 29°19'16.4"E	0.438	golden yellow
WWM-68	Capricorn	Ga-Manamela	23°43'01.7"S 29°14'04.7"E	0.468	brown-olive
WWM-76	Capricorn	Ga-Manamela	23°43'05.1"S 29°14'01.3"E	0.546	cream

STI; stress tolerance index

5.2.2 Fabrication rhizotron prototype

A root rhizotron was fabricated following the method described by Wiese et al. (2005). Transparent Impex Polycarb sheets of 3 mm thickness purchased from Maizey Private Limited, Pietermaritzburg, South Africa, were cut into rectangular sheets (R_1 and R_4) measuring 50 cm in length and 30 cm width using a table saw (Ryobi, Hiroshima, Japan). Wooden boards of 12 mm thickness were cut into rectangular planks measuring 50 cm length and 3 cm width (R_2 and R_3). All the cut rhizotron pieces (R_1, R_2, R_3 and R_4) were held together using a Grip GV9365 Bench Vice (100mm) to allow drilling aligned pilot holes (Figure 5.1).

Holes of 8 mm ⌀ were drilled on each rhizotron piece on a flat surface using Ryobi 16 mm bench drill press. Rhizotron pieces were assembled and secured using an adhesive (NO MORE NAILS, Pattex®), cable ties (T5OI 4.8 × 300 mm) and brown buff packaging tape. Each lateral side of the rhizotron was used to evaluate different systems for non-disruptive visualisation of roots while holding the substrate in place (Figure 5.1). On average, each rhizotron weighs ≈ 0.948 ± 0.038 kg, enclosing ≈ 1.8×10^{-3} m^3 of soil. The estimated cost for a single unit of rhizotron was R 114.30/ US$7.00 (Supplementary File 1).

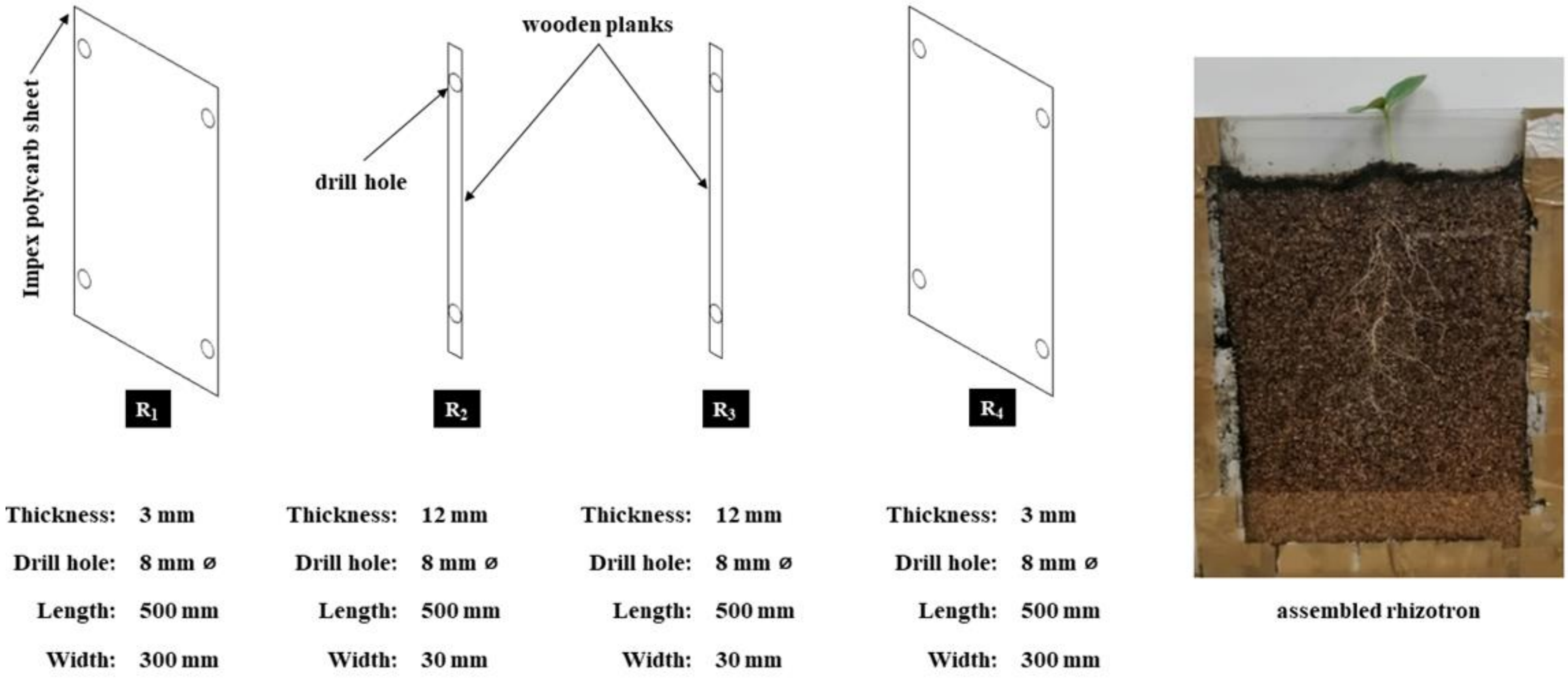

Figure 5.1: Sketch of an inexpensive rhizotron design assembly—observations of the root systems are taken on the lateral sides of the rhizotrons

5.2.3 *Growth substrate selection*

The substrate was selected based on the gravimetric water content (θ_g) of (i) filter sand, (ii) Gromor potting Mix 30 dm^3 (pine bark) and (iii) a mix of Gromor potting Mix 30 dm^3 and filter sand mixed in ratio 1:3. Each of the three substrates was filled in a rhizotron weighing (0.948 ± 0.038 kg). The substrate was transferred into a ceramic bowl and dried in an oven at 105 °C for 24 hours. Mass of dry soil was determined by subtracting the mass of empty rhizotron from the sum mass of oven-dry soil and rhizotron. The substrate in each rhizotron was watered to saturation and left to drain freely through percolation. The change in rhizotron weight was measured daily using a sensitive electron balance (Adam AAA 100L) for 35 days. The (θ_g) of each substrate was calculated according to Haney and Haney (2010) (Equation 1). Based on these results, a mix of Gromor potting Mix 30 dm^3 and filter sand mixed in ratio 1:3 was used for the present study.

$$(\boldsymbol{\theta g})\ (\%) = \left[\frac{Mass\ of\ moist\ soil\ (g)_{(saturated)} - Mass\ of\ oven\ dried\ soil\ (g)}{Mass\ of\ oven\ dried\ soil\ (g)}\right] \times 100 \qquad (1)$$

5.2.4 *Experimental design and growth conditions*

Root rhizotron experiments were done under glasshouse conditions at the Controlled Research Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37′37.5″**S** and 30°24′10.4″**E)**. The glasshouse's mean air temperature and relative humidity were 25 ± 2 °C and 60 ± 3%, respectively. The first rhizotron experiment was conducted between September 2021-October 2021 and the second between October and November 2021. The study was designed as a 9 × 2 factorial experiment with 9 citron watermelon accessions grown under two water regimes: non-stressed (NS) and water-stressed (WS). The experiment was laid in a completely randomized design (CRD) with three replications, giving 54 experimental units (1.8×10^{-3} m^3 rhizotrons). One seed of each accession was sowed in a rhizotron filled with a weed-free Gromor potting Mix 30 dm^3 and filter sand mixed in ratio 1:3. Plants under NS were irrigated at planting, 14 days after planting (DAP) and 28 DAP. For WS treatment, irrigation was done at planting only. Soil moisture curve (Figure 5.3) was used to estimate soil water content throughout the experiment. Each lateral side of the rhizotron was covered with black polyethylene plastic to simulate darkness and avoid light-induced root growth. Two-dimensional root images were captured from 8 DAP to 35 DAP using the method described in Section 5.2.5. Leaf images were also captured to monitor changes in leaf area. The experiment was harvested 35 DAP. The roots and shoots were separated, dried in an oven at 70 °C for 24 hours. A precision scale (UW4200H Shimadzu, Japan) was used to measure root and shoot dry mass.

5.2.5 *Image acquisition*

A camera positioning technique was used to hold the camera at a constant distance (80 cm) from the rhizotron for time-series digital capturing of root growth. Images were captured on both lateral sides of the rhizotron daily from 8 DAP to 35 DAP. An AI Camera of 16 Megapixels (Huawei Y9 Prime 2019) was used to capture images. Camera settings included a resolution of 4:3, assistive grid on, and a timer of 3 s. Images were collected in raw -format with a colour depth of 12 bits and an image size of 4288 × 2848 pixels. Leaf area was measured using the Easy Leaf Area Smartphone application (Heaslon, University of California, California) described by Easlon and Bloom (2014).

5.2.6 *Image analysis and data collection*

A software package (RootSnap Version 1.3.2.25, CID Bio-Science Inc.) analysed 2-dimensional images of plant roots captured from rhizotron laterals. The software performed the predictions in automatic mode with manual corrections (Figure 5.2). Root system architectural traits (Table 5.2) from captured root images were quantified using a user-assisted root image analysis package (RootSnap Version 1.3.2.25, CID Bio-Science Inc.) on a computer tablet (Microsoft Surface). A Microsoft Surface Pro 4 pen/stylus was used to trace the roots. Continuous data from 8 DAP to 35 DAP was collected for root system width (RSW), root system depth (RSD), convex hull area (CHA), total root length (TRL), root branch count (RBC) and leaf area (LA). Other root traits summarised in (Table 5.2) were measured after harvesting the experiment (35 DAP).

Table 5.2: Description of measured traits in citron watermelon accessions grown in a root rhizotron and assessed from 8 DAT to 35 DAT under water stress and non-stress conditions

Trait(s)	Description	Unit(s)
Root system width (RSW)	Maximal horizontal distribution of a root system	cm
Root system depth (RSD)	Maximal vertical depth of a root system	cm
Convex hull area (CHA)	Area of the convex hull that encompasses the root system	cm^2
Total root length (TRL)	Total sum of seminal and lateral root length	cm
Root branch count (RBC)	Number of lateral roots emerging from the primary root	-
Total root volume (TRV)	Total volume of the root system	cm^3
Leaf area (LA)	Area of the leaf	cm^2
Leaf number (LN)	Number of leaves	-
First seminal root length (FSRL)	Length of radicle (measured one day after germination)	cm
Seminal root angle (SRA)	Angle between the outermost left and right seminal roots	°
Root dry mass (RDM)	Total dry mass of roots per plant	g
Shoot dry mass (SDM)	Total dry mass of shoots per plant	g
Root–shoot mass ratio (RSM)	Total root dry mass divided by shoot dry mass	-
Root mass ratio (RMR)	Dry mass of root divided by the total dry mass of entire plant	$g\ g^{-1}$
Shoot mass ratio (SMR)	Dry mass of shoot divided by the total dry mass of entire plant	$g\ g^{-1}$
Root tissue density (RTD)	Total root dry mass divided by root volume	$g\ cm^{-3}$

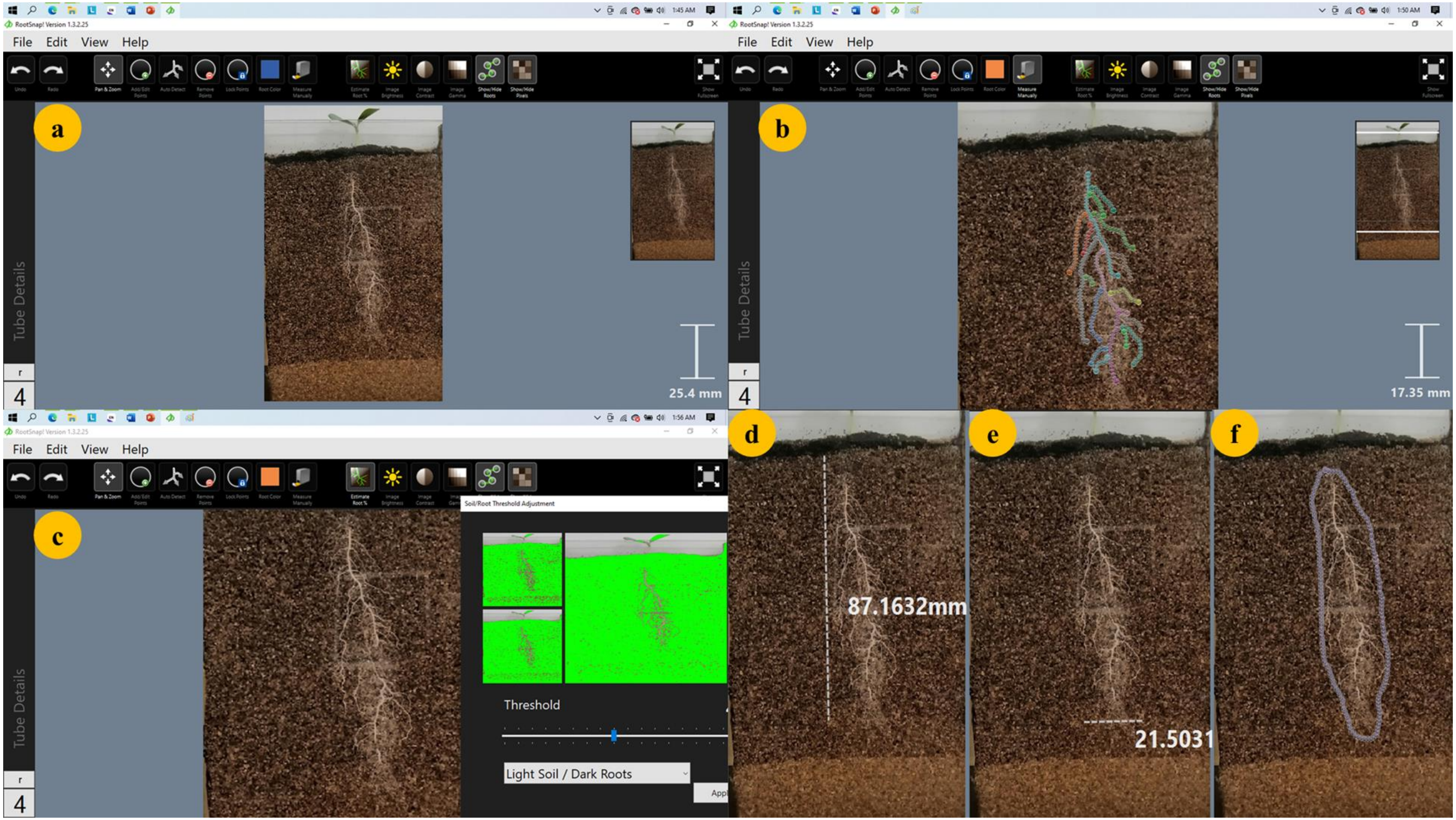

Figure 5.2: Illustration of how RootSnap software was used to analyze and collect data from captured root images (**a**) root image in raw format imported from local storage to RootSnap (**b**) tracing the root using Microsoft Surface Pro 4 stylus to measure total root length (**c**) automated digital image analysis mode (**d**) measurement of root system depth (**e**) measurement of root system width (**f**) measurement of root convex hull area

5.2.7 Statistical analysis

Analysis of variance was performed for traits measured using Genstat 20th Edition (VSN International, Hempstead, United Kingdom). Means were separated using Fisher's protected least significant difference (LSD) test when treatments showed significant effects on measured parameters at 5% level of significance. Principal component analysis (PCA) and the biplot diagrams were exploited using Origin Pro 2021b (OriginLab Corporation). Pearson correlations were computed based on mean values using GraphPad Prism Version 9.2.0 (GraphPad Software, Inc.). Agglomerative Hierarchical Clustering (AHC) was done according to Ward's method using squared Euclidean distance to measure similarity using XLSTAT.

5.3 Results

5.3.1 Gravimetric water

In Figure 5.3, the rate of exponential moisture loss was higher in sand soil (0.131% day^{-1}) compared to a mix of sand-pine bark mix (0.094% day^{-1}). It took nine days for sand soil to lose 60% of the soil water content, while it took 14 days for sand-pine bark mix to lose the same amount (60%) of soil moisture. At 18 days after saturation, the sand soil had reached the permanent wilting point (PWP). It took ten days more for the sand-pine bark mix to reach PWP status (Figure 5.3). Mixing sand with pine bark (Gromor potting Mix 30 dm^3) improved the water holding capacity of sand. Therefore, based on the soil-moisture curve(s) in Figure 5.3, Gromor potting Mix 30 dm^3 and filter sand mixed in ratio 1:3 was used as a growth substrate.

5.3.2 Root growth of citron watermelon accessions under non-stress and water stress conditions

Under NS condition, the average growth rate of RSW was highest in WWM-76 (0.386 cm day^{-1}) and lowest in WWM-15 (0.191 cm day^{-1}) (Figure 5.4a). In Figure 5.4c, higher average growth rates of RSD (≥ 1.045 cm day^{-1}) were recorded in WWM-76, WWM-41(A) and WWM-39, while lower rates (≤ 0.845 cm day^{-1}) were recorded in WWM-68, WWM-15 and WWM-37(2). Convex hull area of the root system increased at a higher rate (≥ 60.933 cm^2 day^{-1}) in WWM-76, WWM-09 and WWM-41(A) compared to WWM-64, WWM-46 and WWM-68 (≤31.715 cm^2 day^{-1}) (Figure 5.4e). The average growth rate of TRL was higher in WWM-39, WWM-37(2), and WWM-41(A) (≥ 2.207 cm day^{-1}) and lower in WWM-15, WWM-46, and WWM-68 (≤ 1.670 cm day^{-1}) (Figure 5.4g). Accessions WWM-09, WWM-41(A) and WWM-76 had average RBC ≈ 2 branches day^{-1}, while WWM-15, WWM-37(2), WWM-39 and WWM-68 were forming approximately one branch per day (Figure 5.4i). Accessions WWM-09, WWM-37(2) and WWM-41(A) had higher leaf area expansion rates ≥ 1.987 cm^2 day^{-1}.

Lower leaf expansion rates (≤ 1.731 cm^2 day^{-1}) were recorded in WWM-39, WWM-46 and WWM-68 (Figure 5.4k).

Under WS condition, the average growth rate of RSW was higher in WWM-76, WWM-41(A) and WWM-37(2) (≥ 0.325 cm day^{-1}) and lower in WWM-64, WWM-15 and WWM-68 (≤ 0.284 cm day^{-1}) (Figure 5.4b). In Figure 5.4d, higher average growth rates of RSD (≥ 1.152 cm day^{-1}) were recorded in WWM-09, WWM-41(A) and WWM-76, while lower rates (≤ 0.889 cm day^{-1}) were recorded in WWM-15, WWM-46 and WWM-68. Convex hull area of the root system increased at a higher rate (≥ 78.593 cm^2 day^{-1}) in WWM-76, WWM-41(A) and WWM-09 compared to WWM-15, WWM-46, WWM-64 and WWM-68 (≤ 41.477 cm^2 day^{-1}) (Figure 5.4f). The average growth rate of TRL was higher in WWM-09, WWM-37(2), WWM-41(A) and WWM-76 (≥ 2.207 cm day^{-1}) and lower in WWM-15, WWM-46, WWM-68 and WWM-64 (≤ 1.779 cm day^{-1}) (Figure 5.4 h). WWM-41(A), WWM-68 and WWM-76 had higher leaf area expansion rates ≥ 0.804 cm^2 day^{-1}. Lower leaf expansion rates (≤ 0.403 cm^2 day^{-1}) were recorded in WWM-15, WWM-46 and WWM-64 (Figure 5.4l). From 21 DAP, the leaf area growth curves under WS condition started to plateau and decline in accessions such as WWM-15, WWM-46 and WWM-68 due to water deficit (Figure 5.4l).

Water stress increased the mean growth rate of RSW (0.298 cm day^{-1}) compared to NS condition (0.273 cm day^{-1}) (Figure 5.4a). Both RSD (Figure 5.4b) and CHA (Figure 5.4c) average growth rates for all evaluated accessions were higher under WS condition (1.102 cm day^{-1} and 60.276 cm^2 day^{-1}) than NS conditions (0.909 cm day^{-1} and 48.044 cm^2 day^{-1}) respectively. Mean total root length growth rate did not differ significantly among the accessions under both NS and WS conditions; the rates of TRL growth ranged between 1.670 – 2.207 cm day^{-1} under NS and between 1.779 – 2.325 cm day^{-1} under WS condition (Figure 5.4d). Average rate of root branch count (≈ 1 branch day^{-1}) did not differ between water treatments (Figure 5.4e). In Figure 5.4f, the average leaf area expansion rate was higher under NS condition (1.909 cm^2 day^{-1}) than WS condition (0.762 cm^2 day^{-1}).

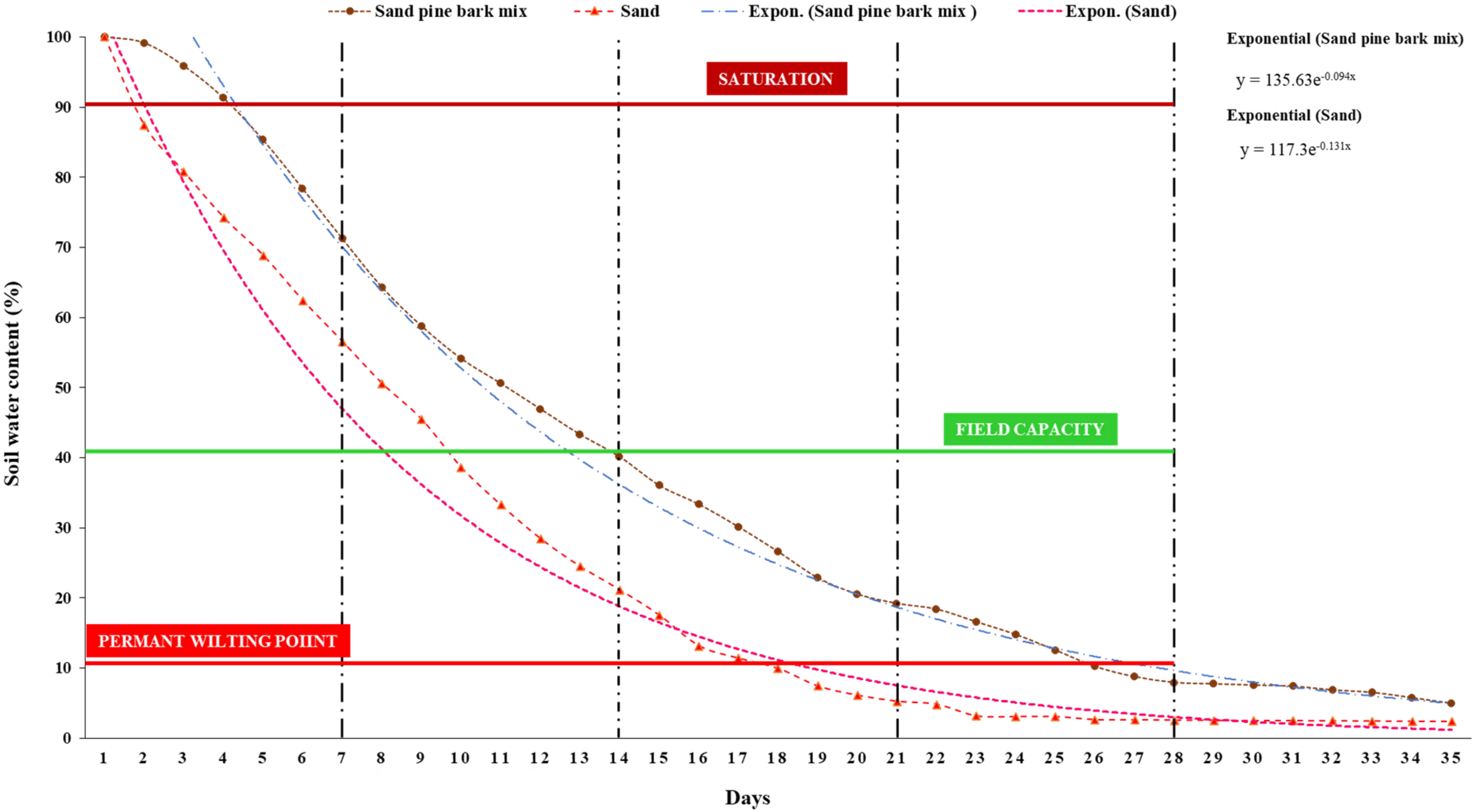

Figure 5.3: Percentage of soil water content depleted versus time (days) in sand soil, pine bark and sand pine bark mix

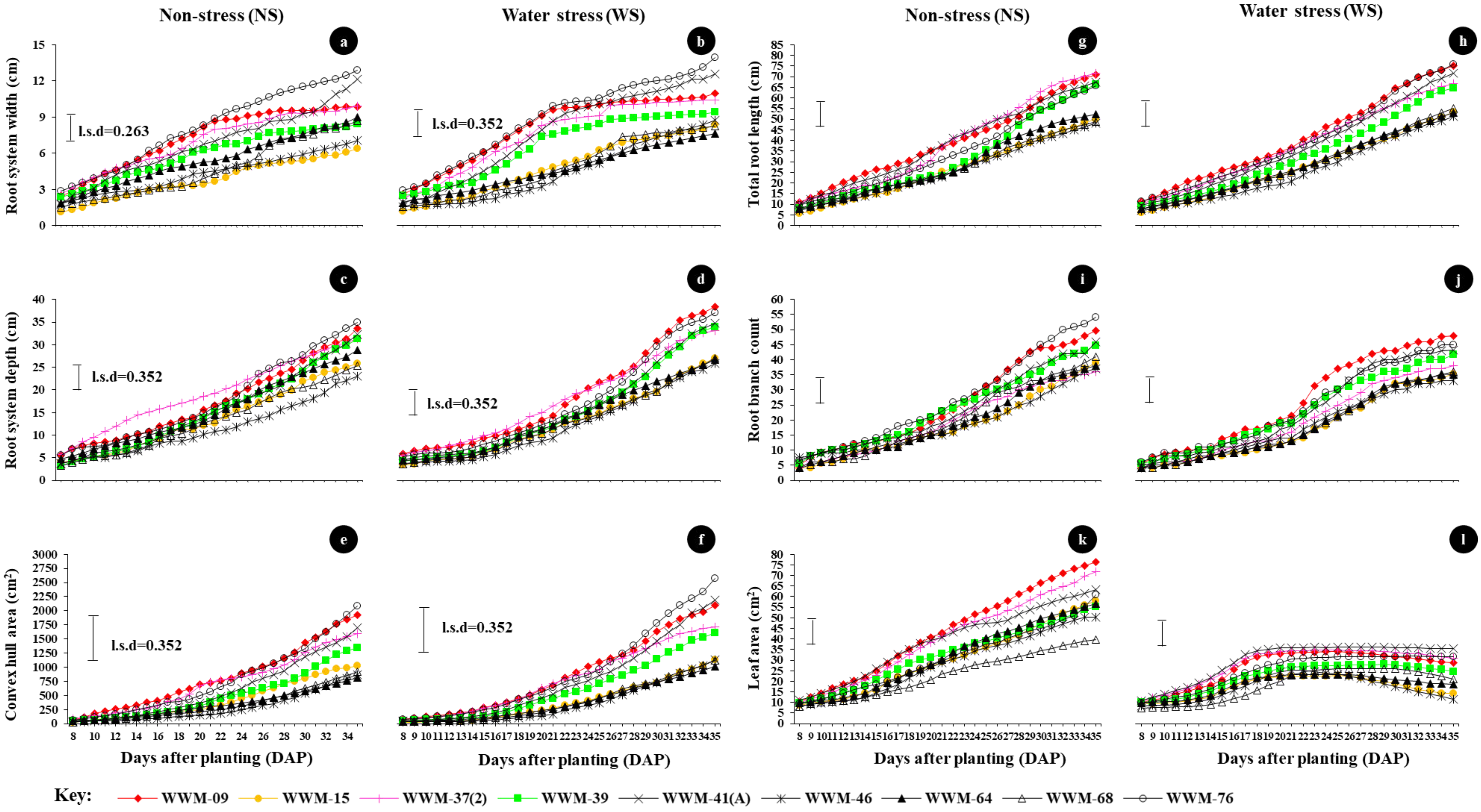

Figure 5.4: Changes in root growth and leaf area of nine drought-tolerant citron watermelon accessions under non-stressed and water stress conditions from 8 to 35 days after planting

Table 5.3: Average daily growth rates of root traits and leaf area measured in 9 citron watermelon accessions from 8 DAP to 35 DAP under non-stress and water-stress conditions

	RSW (cm day $^{-1}$)		**RSD** (cm day $^{-1}$)		**CHA** (cm^{2} day $^{-1}$)		**TRL** (cm day $^{-1}$)		**RBC** (branch/day)		**LA** (cm^{2} day $^{-1}$)	
Accession	**NS**	**WS**	**NS**	**WS**	**NS**	**WS**	**NS**	**WS**	**NS**	**WS**	**NS**	**WS**
WWM-09	0.285^{c}	0.314^{d}	1.016^{b}	1.278^{a}	66.743^{ab}	78.593^{b}	2.192^{bc}	2.325^{b}	1.746^{f}	1.800^{e}	2.578^{a}	0.762^{b}
WWM-15	0.191^{h}	0.284^{g}	0.859^{c}	0.889^{d}	39.172^{e}	40.728^{e}	1.670^{e}	1.779^{d}	1.263^{b}	1.298^{b}	1.909^{d}	0.278^{e}
WWM-37(2)	0.273^{e}	0.325^{c}	0.909^{c}	1.102^{c}	58.729^{c}	68.341^{c}	2.499^{a}	2.207^{c}	1.293^{bc}	1.432^{c}	2.379^{b}	0.788^{b}
WWM-39	0.236^{g}	0.298^{e}	1.047^{ab}	1.141^{bc}	48.044^{d}	60.276^{d}	2.207^{bc}	2.144^{c}	1.475^{de}	1.482^{c}	1.731^{e}	0.667^{c}
WWM-41(A)	0.338^{b}	0.430^{a}	1.045^{ab}	1.152^{bc}	60.933^{bc}	79.814^{b}	2.228^{b}	2.362^{ab}	1.507^{e}	1.677^{d}	1.987^{c}	0.896^{a}
WWM-46	0.189^{h}	0.296^{e}	0.693^{d}	0.875^{d}	29.129^{f}	40.474^{e}	1.557^{f}	1.724^{d}	1.085^{a}	1.197^{a}	1.745^{e}	0.277^{e}
WWM-64	0.252^{f}	0.221^{h}	0.908^{c}	0.896^{d}	27.663^{f}	37.138^{e}	1.783^{d}	1.745^{d}	1.322^{c}	1.317^{b}	1.950^{cd}	0.403^{d}
WWM-68	0.280^{d}	0.292^{f}	0.845^{c}	0.877^{d}	31.715^{f}	41.477^{e}	1.553^{f}	1.779^{d}	1.453^{d}	1.319^{b}	1.268^{f}	0.804^{b}
WWM-76	0.386^{a}	0.403^{b}	1.120^{a}	1.250^{ab}	71.403^{a}	91.609^{a}	2.139^{c}	2.463^{a}	1.832^{g}	1.615^{d}	1.783^{e}	0.894^{a}
Mean	0.270	0.318	0.938	1.051	48.170	59.828	1.981	2.057	1.442	1.460	1.926	0.641
LSD	3.431×10^{-3}	0.003	0.086	0.120	6.124	7.376	0.086	0.100	0.051	0.069	0.067	0.051
CV (%)	0.700	0.600	5.300	6.700	7.400	7.200	2.500	2.900	2.100	2.700	2.100	4.700
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Values in the same column followed by the same letter are not significantly different, while values with different superscript letters are significantly different according to Fisher's test. **RSW**; root system width, **RSD**; root system depth, **CHA**; convex hull area, **TRL**; total root length, **RBC**; root branch count, **LA**; leaf area *Average growth rate is the coefficient of *x* in the linear equation ($y = mx + c$) derived from linear graphs in Figure 5.4

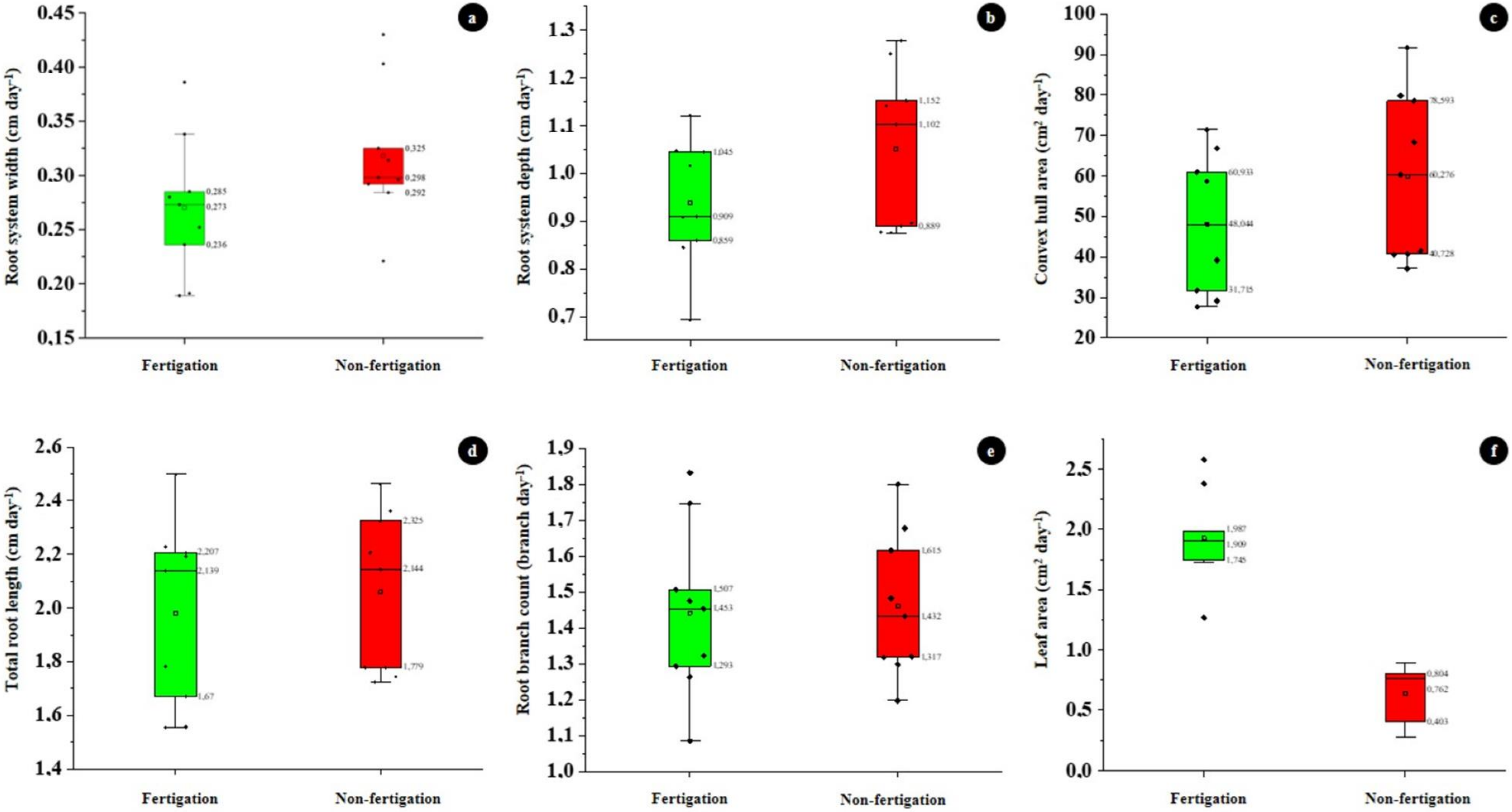

Figure 5.5: Growth rate comparison of root traits and leaf area under non-stress and water stress conditions (**a**) root system width, (**b**) root system depth, (**c**) convex hull area, (**d**) total root length, (**e**) root branch count and (**f**) leaf area

5.3.3 ANOVA showing accession, water regime and their interactions on root and shoot traits of citron watermelon

ANOVA for evaluated root traits indicated that the effects of irrigation, genotype, and interaction were significantly different for most traits (Table 5.4). Water stress significantly increased average RSW, RSD, CHA, TRV, RSM and RMR compared to non-stress condition (Figure 5.6a, b, c, f, m and n). Accessions WWM-09, WWM-41(A) and WWM-76 recorded RSW values of ≥ 10.940 cm under WS condition (Table 5.6), whereas accessions WWM-68, WWM-39, WWM-15 and WWM-46 recorded RSW values of ≤ 8.644 cm under NS condition (Table 5.5).

For RSD, accessions WWM-09, WWM-41(A) and WWM-76 recorded values ≥ 34.820 cm under WS, compared to WWM-15, WWM-46, WWM-68 and WWM-64, which recorded RSD values ≤ 28.770 cm under NS condition. The mean CHA for evaluated accessions was significantly higher (1620.111 cm^2) under WS condition compared to NS condition (1362.566 cm^2) (Figure 5.6c). For TRV, WWM-09, WWM-39, WWM-41(A), WWM-37(2), WWM-76 and WWM-68 recorded values ≥ 1.928 cm^3 under WS, compared to WWM-15 and WWM-46 which recorded TRV values ≤ 1.541 cm^3 under NS condition.

Mean root–shoot mass ratio for evaluated genotypes was significantly higher (1.8881) under WS condition compared to NS condition (1.2343) (Figure 5.6m). Mean RMR for evaluated accessions was significantly higher (0.6463 g g^{-1}) under WS condition compared to NS condition (0.5463 g g^{-1}) (Figure 5.6n). Water stress significantly reduced mean RBC, LA, LN, RDM, SDM, SMR and RTD among the evaluated accessions compared to NS condition (Figure 5.6e, i, j, k, l, o and p). The mean RBC for evaluated accessions was higher (43 branches) under NS condition compared to WS condition (39 branches) (Figure 5.6e). Average leaf number for evaluated accessions was significantly higher (8 leaves) under NS condition compared to WS condition (5 leaves) (Figure 5.6j).

For RDM, accessions WWM-41(A), WWM-76, WWM-39 and WWM-09 recorded values ≥ 2.695 g under NS, compared to WWM-15, WWM-37(2), WWM-46 and WWM-68 which recorded RDM ≤ 1.355 g under WS condition. WWM-09, WWM-39, WWM-64, WWM-41(A), WWM-46and WWM-76 recorded SDM values of ≥ 2.172 g under NS condition, whereas accessions WWM-15, WWM-37(2) and WWM-76 recorded SDM values ≤ 0.747 g under WS condition. Under NS condition, higher SMR (≥ 0.514 g g^{-1}) was recorded in WWM-

15 and WWM-64, whereas accessions WWM-09 and WWM-76 recorded lower SMR (≤ 0.392 g g^{-1}).

Under WS condition, accessions WWM-15, WWM-64 and WWM-68 recorded higher SMR (≥ 0.387 g g^{-1}) and accessions WWM-09 and WWM-76 recorded lower values (≤ 0.285 g g^{-1}). The mean root tissue density (RTD) for evaluated accessions was significantly higher (1.7252 g cm^3) under NS condition compared to WS condition (0.8043 g cm^3) (Figure 5.6p).

Table 5.4: Analysis of variance showing mean squares and significant tests for root and leaf of 9 citron watermelon landrace accessions evaluated under non-stressed and water-stressed conditions

Source of variation	**d.f**	**RSW**	**RSD**	**CHA**	**TRL**	**RBC**	**TRV**	**LA**	**LN**
Accession (A)	8	25.763^{**}	117.558^{**}	$1.640\times10^{6**}$	578.406^{**}	183.560^{**}	1.060^{**}	$4.383\times10^{2**}$	6.292
Water Condition (WC)	1	6.001^{**}	48.964^{**}	$8.952\times10^{5**}$	106.145^{**}	150^{**}	2.212^{**}	$1.665\times10^{4**}$	280.167^{*}
A × WC	8	1.545^{**}	5.107^{**}	$3.152\times10^{4**}$	29.877^{**}	12.625^{**}	0.163^{**}	$1.255\times10^{2**}$	3.792^{*}
Residual	36	0.025	0.051	131.900	0.018	0.093	0.023	4.138×10^{-2}	1.847

Source of variation	d.f	FSRL	SRA	RDM	SDM	RSM	RMR	SMR	RTD
Accession (A)	8	1.892^{**}	141.800^{ns}	2.875^{**}	0.296^{**}	0.693^{**}	0.015^{**}	0.015^{**}	0.520^{**}
Water Condition (WC)	1	0.056	197.100^{ns}	22.970^{**}	27.549^{**}	5.771^{**}	0.135^{**}	0.135^{**}	11.447^{**}
A × WC	8	0.048^{**}	130.400^{*}	1.443^{**}	0.330^{**}	0.073^{*}	8.489×10^{-4}	$8.489\times10^{-4**}$	0.388^{**}
Residual	36	0.007	379.700	0.056	0.016	0.027	5.185×10^{-4}	5.185×10^{-4}	0.045

d f; degrees of freedom, **RSW**; root system width, **RSD**; root system depth, **TRL**; total root volume, **RBC**; root branch count, **TRV**; total root volume, **LA**; leaf area, **LN**; leaf number, **FSRL**; first seminal root length, **SRA**; seminal root angle, **RDM**; root dry mass, **SDM**; shoot dry mass, **RSM**; root shoot mass ratio, **RMR**; root mass ratio, **SMR**; shoot mass ratio, **RTD**; root tissue density. ***** and ****** denote significant at 5 and 1% probability levels, respectively. **ns**, non-significant

Table 5.5: Mean values for root and leaf traits of 9 citron watermelon accessions evaluated in rhizotrons under non-stressed condition at 35 days after planting

	Below ground								**Above ground**		**Allometry**					
Accession	RSW	RSD	CHA	TRL	RBC	TRV	FSRL	SRA	LA	LN	RDM	SDM	RSM	RMR	SMR	RTD
WWM-09	9.875^{c}	33.620^{b}	1928^{b}	70.990^{b}	50^{b}	1.753^{bc}	2.458^{bc}	65.470^{a}	76.410^{a}	11.000^{ab}	4.489^{b}	2.884^{a}	1.553^{ab}	0.608^{ab}	0.392^{ef}	2.584^{a}
WWM-15	6.411^{g}	25.860^{f}	1020^{f}	49.970^{g}	39^{f}	1.541^{cd}	1.106^{f}	60.600^{a}	57.880^{e}	7.000^{e}	1.852^{e}	2.042^{bc}	0.905^{g}	0.475^{f}	0.525^{a}	1.219^{de}
WWM-37(2)	9.889^{c}	31.260^{d}	1594^{d}	71.720^{a}	36^{i}	1.837^{b}	2.611^{b}	62.430^{a}	71.820^{b}	9.000^{cd}	2.061^{de}	1.800^{c}	1.142^{def}	0.533^{de}	0.467^{bc}	1.137^{e}
WWM-39	8.448^{e}	31.330^{d}	1337^{e}	66.480^{d}	45^{d}	1.596^{bcd}	2.353^{bc}	45.470^{a}	55.240^{g}	9.000^{de}	2.735^{c}	2.172^{b}	1.266^{cd}	0.558^{cd}	0.442^{cd}	1.717^{cd}
WWM-41(A)	12.132^{b}	32.350^{c}	1702^{c}	67.520^{c}	46^{c}	1.809^{b}	1.724^{d}	66.600^{a}	63.470^{c}	10.000^{bc}	2.695^{c}	2.291^{b}	1.169^{de}	0.538^{de}	0.462^{bc}	1.502^{cde}
WWM-46	7.053^{f}	23.040^{g}	872^{h}	48.060^{i}	37^{h}	1.265^{e}	1.793^{d}	53.030^{a}	50.330^{h}	10.000^{bc}	2.508^{cd}	1.844^{c}	1.370^{bc}	0.577^{bc}	0.423^{de}	1.992^{bc}
WWM-64	8.958^{d}	28.770^{e}	812^{h}	52.140^{f}	38^{g}	1.369^{de}	1.884^{d}	45.300^{a}	56.730^{f}	9.000^{cd}	2.129^{cde}	2.247^{b}	0.950^{fg}	0.486^{f}	0.514^{a}	1.555^{cde}
WWM-68	8.644^{de}	25.420^{f}	920^{g}	48.750^{h}	41^{e}	1.596^{bcd}	1.520^{e}	61.870^{a}	39.870^{i}	11.000^{ab}	2.343^{cde}	2.224^{b}	1.050^{efg}	0.512^{ef}	0.488^{ab}	1.466^{cde}
WWM-76	12.879^{a}	34.910^{a}	2078^{a}	65.660^{e}	54^{a}	2.193^{a}	2.837^{a}	56.970^{a}	60.720^{d}	12.000^{a}	5.118^{a}	3.007^{a}	1.704^{a}	0.630^{a}	0.370^{f}	2.355^{ab}
l.s.d	0.367	0.496	16.730	0.161	0.467	0.228	0.155	33.710	0.483	1.715	0.560	0.288	0.185	0.037	0.037	0.481
CV (%)	2.300	1.000	0.700	0.200	0.600	8.000	4.500	34.200	0.500	10.300	11.300	7.400	8.700	4.000	4.800	16.300
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.842	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Means in the same column followed by the same letter are not significantly different, while values with different superscript letters are significantly different according to Fisher's test. **RSW**; root system width (cm), **RSD**; root system depth (cm), **CHA**; convex hull area (cm^{2}), **TRL**; total root length (cm), **RBC**; root branch count, **TRV**; total root volume (cm^{3}), **FSRL**; first seminal root length (cm), **SRA**; seminal root angle, **LA**; leaf area (cm^{2}), **LN**; leaf number **RDM**; root dry mass (g), **SDM**; shoot dry mass (g), **RSM**; root–shoot mass ratio, **RMR**; root mass ratio (g g^{-1}), **SMR**; shoot mass ratio (g g^{-1}), **RTD**; root tissue density (g cm^{-3})

Table 5.6: Mean values for root and leaf traits of 9 citron watermelon accessions evaluated in rhizotrons under water-stress condition at 35 days after planting

	Below ground								**Above ground**		**Allometry**					
Accession	RSW	RSD	CHA	TRL	RBC	TRV	FSRL	SRA	LA	LN	RDM	SDM	RSM	RMR	SMR	RTD
WWM-09	10.940^{c}	38.410^{a}	2102^{c}	75.230^{a}	48^{a}	2.423^{b}	2.618^{b}	58.120^{a}	28.600^{d}	6.000^{ab}	2.097^{a}	0.822^{cd}	2.558^{a}	0.718^{a}	0.281^{e}	0.866^{bc}
WWM-15	8.350^{g}	27.050^{f}	1129^{f}	53.250^{g}	35^{g}	1.450^{d}	1.163^{f}	58.470^{a}	14.170^{h}	4.000^{c}	0.989^{e}	0.711^{de}	1.393^{d}	0.582^{e}	0.418^{a}	0.683^{cde}
WWM-37(2)	10.400^{d}	33.030^{e}	1718^{d}	66.510^{d}	38^{e}	2.697^{ab}	2.285^{c}	71.700^{a}	30.740^{c}	4.000^{c}	1.355^{d}	0.747^{de}	1.827^{bc}	0.645^{bcd}	0.355^{bcd}	0.504^{e}
WWM-39	9.450^{e}	34.010^{d}	1606^{e}	64.720^{e}	42^{d}	1.983^{c}	2.483^{b}	61.900^{a}	24.690^{e}	5.000^{bc}	1.916^{b}	0.960^{b}	2.005^{b}	0.666^{b}	0.334^{d}	0.975^{b}
WWM-41(A)	12.600^{b}	34.820^{c}	2193^{b}	71.730^{c}	43^{c}	2.401^{b}	1.899^{d}	55.670^{a}	35.430^{a}	5.000^{bc}	1.747^{c}	0.900^{bc}	1.976^{b}	0.663^{bc}	0.338^{cd}	0.733^{cd}
WWM-46	8.720^{f}	25.820^{h}	1126^{f}	51.180^{h}	33^{h}	1.315^{d}	1.531^{e}	65.670^{a}	11.360^{i}	5.000^{bc}	1.258^{d}	0.771^{cde}	1.648^{bcd}	0.620^{cde}	0.380^{abc}	0.969^{b}
WWM-64	7.620^{h}	26.620^{g}	1014^{g}	53.030^{g}	35^{g}	1.493^{d}	1.849^{d}	55.670^{a}	18.600^{g}	7.000^{a}	1.888^{b}	1.293^{a}	1.466^{cd}	0.594^{e}	0.407^{a}	1.279^{a}
WWM-68	8.270^{g}	26.950^{f}	1114^{f}	55.070^{f}	36^{f}	1.928^{c}	1.570^{e}	63.030^{a}	21.070^{f}	5.000^{bc}	1.233^{d}	0.780^{cde}	1.585^{cd}	0.613^{de}	0.387^{ab}	0.641^{de}
WWM-76	13.940^{a}	36.990^{b}	2579^{a}	75.800^{a}	45^{b}	2.912^{a}	2.888^{a}	61.900^{a}	31.730^{b}	5.000^{bc}	1.709^{c}	0.681^{e}	2.535^{a}	0.716^{a}	0.285^{e}	0.589^{de}
l.s.d	0.120	0.236	22.280	0.278	0.572	0.292	0.137	33.130	0.100	1.715	0.122	0.120	0.354	0.041	0.041	0.180
CV (%)	0.700	0.400	0.800	0.300	0.800	8.200	3.900	31.500	0.200	19.600	4.500	8.200	10.900	3.700	6.700	13.100
P-value	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.984	<.001	0.045	<.001	<.001	<.001	<.001	<.001	<.001

Means in the same column followed by the same letter are not significantly different, while values with different superscript letters are significantly different according to Fisher's test. **RSW**; root system width (cm), **RSD**; root system depth (cm), **CHA**; convex hull area (cm^{2}), **TRL**; total root length (cm), **RBC**; root branch count, **TRV**; total root volume (cm^{3}), **FSRL**; first seminal root length (cm), **SRA**; seminal root angle, **LA**; leaf area (cm^{2}), **LN**; leaf number **RDM**; root dry mass (g), **SDM**; shoot dry mass (g), **RSM**; root–shoot mass ratio, **RMR**; root mass ratio (g g^{-1}), **SMR**; shoot mass ratio (g g^{-1}), **RTD**; root tissue density (g cm^{-3})

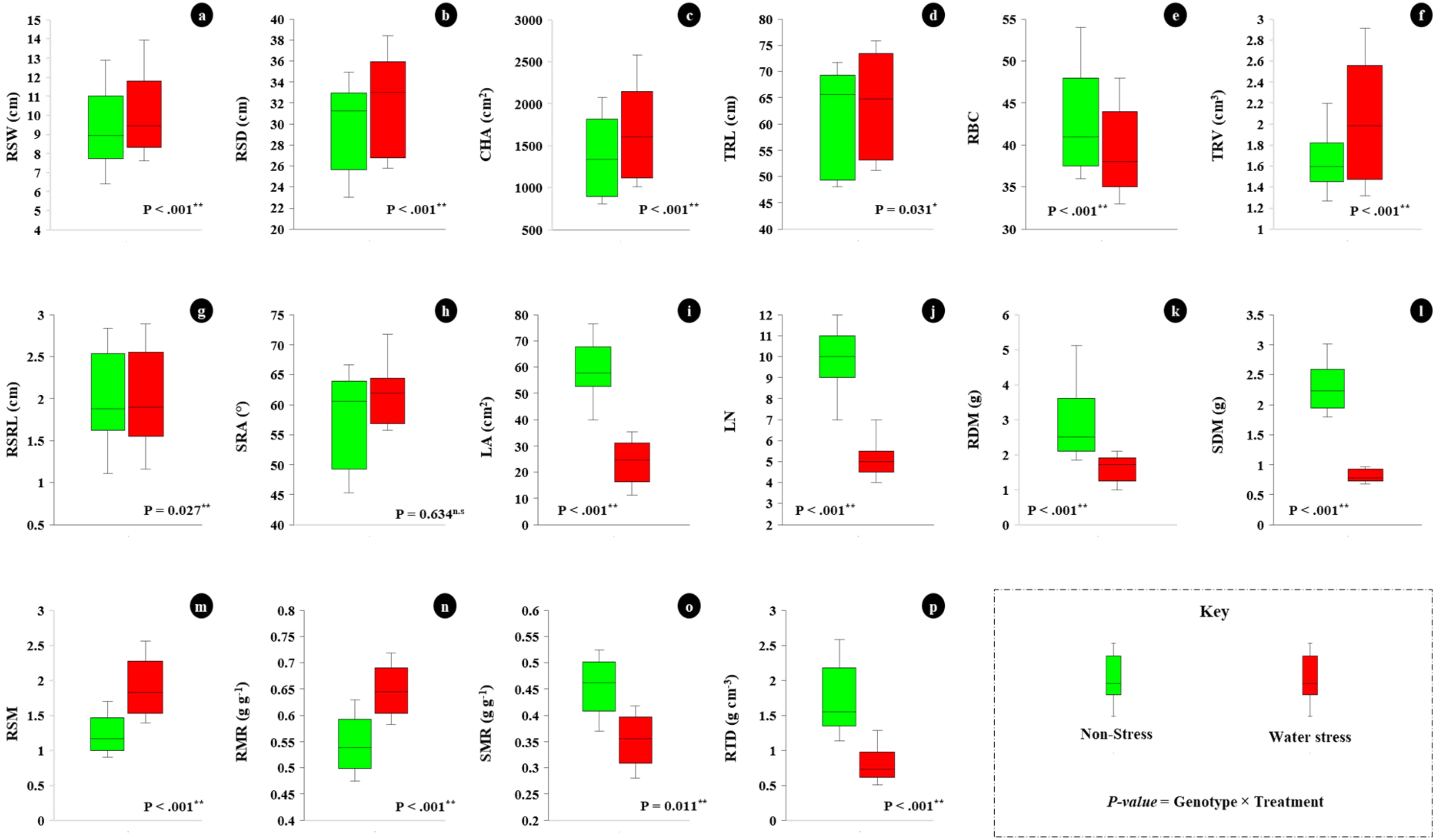

Figure 5.6: Summarized effect of non-water stress and water stress at 35 DAP: (**a**) root system width, (**b**) root system depth, (**c**) convex hull area, (**d**) total root length, (**e**) root branch count, (**f**) total root volume, (**g**) first seminal root length, (**h**) seminal root angle, (**i**) leaf area, (**j**) leaf number, (**k**) root dry mass, (**l**) shoot dry mass, (**m**) root shoot mass ratio, (**n**) root mass ratio, (**o**) shoot mass ratio (**p**) root tissue density

5.3.4 Pearson correlation analysis showing associations of RSA traits among citron watermelon accessions under non-stressed and water-stressed conditions

Pearson correlation coefficients showing evaluated traits relationships among citron watermelon accessions under non-stress and water stress conditions are presented in Table 5.7. Under NS condition, significant and positive correlations were observed between RDM and RBC ($r = 0.91$; $p = 0.002$), SDM ($r = 0.91$; $p = 0.001$), RSM ($r = 0.92$; $p < 0.001$), RMR ($r = 0.88$; $p = 0.021$) and RTD ($r = 0.89$; $p = 0.019$). Root branch count was positively correlated with SDM ($r = 0.92$; $p < 0.001$). Significant and negative correlations were observed between SMR with RTD ($r = -0.86$; $p = 0.017$), RSM ($r = -0.99$; $p < 0.001$), SDM ($r = -0.63$; $p = 0.048$), RDM ($r = -0.88$; $p = 0.016$), RBC ($r = -0.73$; $p = 0.031$) and CHA ($r = -0.72$; $p = 0.045$) (Table 5.7; *bottom diagonal*).Under water stress condition, significant and positive correlations were observed between RSW with RSD ($r = 0.83$; $p < 0.001$), CHA ($r = 0.98$; $p < 0.001$), TRL ($r = 0.90$; $p < 0.01$), TRV ($r = 0.85$; $p = 0.021$), RSM ($r = 0.82$; $p = 0.033$) and RMR ($r = 0.83$; $p = 0.001$). Significant and negative correlations were observed between SMR with RSW ($r = -0.82$; $p = 0.027$), RSD ($r = -0.94$; $p = 0.001$), CHA ($r = -0.91$; $p < 0.001$), RDM ($r = -0.65$; $p = 0.042$), RMR ($r = -1.000$; $p < 0.001$), TRL ($r = -0.93$; $p = 0.009$), RBC ($r = -0.93$; $p = 0.008$), TRV ($r = -0.78$; $p = 0.032$), FSRL ($r = -0.90$; $p < 0.001$), LA ($r = -0.73$; $p = 0.027$) and RSM ($r = -0.99$; $p < 0.001$) (Table 5.7; *top diagonal*).

5.3.5 Principal component analysis (PCA) for root system architecture of citron watermelon accessions evaluated under non-stressed and water-stressed conditions

Table 5.8 shows PCA with factor loadings, eigenvalues, and percent variance of the evaluated RSA traits of nine selected drought-tolerant accessions under non-stressed and water-stressed conditions. Under NS condition, PC1 accounted for 63.95% of the total variation and was positively correlated with RSW, RSD, TRV, RDM CHA, RMR and RTD. PC2 positively correlated with TRL, LA and SRA, contributing 14.81% of the total variation. Under WS condition, PC1 accounted for 64.50% of the total variation and was positively correlated with RSD, RSW, CHA, TRL, RBC, TRV, FSRL, RMR, RSM, LA, RSM and RMR. Leaf number, RDM, SDM and RTD were positively correlated with PC2, which accounted for 22.85% of the total variation. (Table 5.8).

The PC biplots based on PCA analysis were used to visualize the relationship between citron watermelon accessions and root and leaf traits under NS (Figure 5.7a) and WS conditions (Figure 5.7b). Traits represented by parallel vectors or close to each other revealed a strong positive association, and those located nearly opposite (at 180°) showed a highly negative association, while the vectors toward sides expressed a weak relationship. Under NS condition, accessions WWM-09, WWM-39 and WWM-76 are grouped based on high RBC, SDM, RSM, LN and RTD. Accessions WWM-37(2) and WWM-15 are grouped based on high SMR (Figure 5.7a). Under WS conditions, accessions WWM-09, WWM-39 and WWM-41(A) are grouped based on high RBC, RDM, RSM, RSD, RMR and FSRL. Accessions WWM-37(2) and WWM-76 are grouped based on high TRL, LA, CHA, RSW and TRV. WWM-46, WWM-68 and WWM-15 are grouped based on high SMR (Figure 5.7b).

5.3.6 Root vigour (foraging capacity) of citron watermelon accessions under water stress condition

Agglomerative hierarchical clustering for means of root traits under water stress condition at 35 DAP (Table 5.6), classified the nine landrace accessions into four groups (Figure 5.8). Group A (high root foraging) comprised one accession (WWM-76). Group B comprised two accessions (WWM-09 and WWM-41(A)) with moderate-high root foraging. Similarly, group C had two accessions (WWM-37(2) and WWM-39) with moderately low root foraging. Group D (low root foraging) is comprised of four accessions (WWM-15, WWM-46, WWM-64 and WWM-68) (Figure 5.8).

Table 5.7: Pearson correlation coefficients for evaluated traits (root system architectural traits, leaf traits, allometry) under non-stressed condition (*bottom diagonal*) and water stress condition (*top diagonal*)

Traits	**RSW**	**RSD**	**CHA**	**TRL**	**RBC**	**TRV**	**FSRL**	**SRA**	**LA**	**LN**	**RDM**	**SDM**	**RSM**	**RMR**	**SMR**	**RTD**
RSW	**1**	0.83^{**}	0.98^{**}	0.90^{**}	0.78^{**}	0.85^{**}	0.69^{*}	-0.02^{ns}	0.82^{**}	-0.16^{ns}	0.37^{ns}	-0.39^{ns}	0.82^{**}	0.83^{**}	-0.82^{**}	-0.49^{ns}
RSD	0.83^{**}	**1**	0.93^{**}	0.99^{**}	0.97^{**}	0.86^{**}	0.87^{**}	-0.06^{ns}	0.85^{**}	0.01^{ns}	0.66^{*}	-0.22^{ns}	0.93^{**}	0.94^{**}	-0.94^{**}	-0.31^{ns}
CHA	0.80^{**}	0.90^{**}	**1**	0.97^{**}	0.89^{**}	0.89^{**}	0.79^{**}	-0.06^{ns}	0.86^{**}	-0.08^{ns}	0.51^{*}	-0.33^{ns}	0.90^{**}	0.91^{**}	-0.90^{**}	-0.43^{ns}
TRL	0.67^{*}	0.89^{**}	0.87^{**}	**1**	0.95^{**}	0.91^{**}	0.84^{**}	-0.06^{ns}	0.91^{**}	-0.02^{ns}	0.61^{*}	-0.25^{ns}	0.92^{**}	0.93^{**}	-0.93^{**}	-0.39^{ns}
RBC	0.70^{*}	0.75^{**}	0.79^{**}	0.53^{*}	**1**	0.77^{**}	0.82^{**}	-0.25^{ns}	0.79^{**}	0.14^{ns}	0.72^{*}	-0.15^{ns}	0.93^{**}	0.93^{**}	-0.93^{**}	-0.21^{ns}
TRV	0.83^{**}	0.82^{**}	0.89^{**}	0.70^{*}	0.73^{*}	**1**	0.79^{**}	0.23^{ns}	0.92^{**}	-0.19^{ns}	0.38^{ns}	-0.36^{ns}	0.78^{**}	0.80^{**}	-0.80^{**}	-0.62^{*}
FSRL	0.62^{*}	0.76^{**}	0.72^{*}	0.76^{**}	0.51^{*}	0.63^{*}	**1**	0.12^{ns}	0.71^{*}	0.20^{ns}	0.73^{*}	-0.04^{ns}	0.89^{**}	0.90^{**}	-0.90^{**}	-0.11^{ns}
SRA	0.32^{ns}	0.20^{ns}	0.47^{ns}	0.31^{ns}	0.21^{ns}	0.46^{ns}	-0.09^{ns}	**1**	-0.02^{ns}	-0.58^{*}	-0.43^{ns}	-0.50^{*}	-0.03^{ns}	0.01^{ns}	-0.02^{ns}	-0.48^{ns}
LA	0.41^{ns}	0.70^{*}	0.71^{*}	0.80^{**}	0.30^{ns}	0.46^{ns}	0.54^{*}	0.38^{ns}	**1**	-0.05^{ns}	0.53^{*}	-0.10^{ns}	0.70^{*}	0.74^{*}	-0.73^{*}	-0.44^{ns}
LN	-0.07^{ns}	0.22^{ns}	0.06^{ns}	0.28^{ns}	0.23^{ns}	-0.02^{ns}	0.30^{ns}	-0.54^{*}	-0.14^{ns}	**1**	0.70^{*}	0.79^{ns}	0.12^{ns}	0.09^{ns}	-0.09^{ns}	0.80^{**}
RDM	0.64^{*}	0.68^{*}	0.79^{**}	0.49^{ns}	0.91^{**}	0.68^{*}	0.67^{*}	0.18^{ns}	0.38^{ns}	0.07^{ns}	**1**	0.53^{*}	0.65^{*}	0.66^{*}	-0.65^{*}	0.46^{ns}
SDM	0.64^{*}	0.69^{*}	0.68^{*}	0.39^{ns}	0.92^{**}	0.63^{*}	0.47^{ns}	0.18^{ns}	0.31^{ns}	0.01^{ns}	0.91^{**}	**1**	-0.28^{ns}	-0.28^{ns}	0.28^{ns}	0.84^{**}
RSM	0.54^{*}	0.55^{*}	0.73^{*}	0.50^{*}	0.76^{**}	0.56^{*}	0.74^{*}	0.11^{ns}	0.35^{ns}	0.16^{ns}	0.92^{**}	0.69^{*}	**1**	0.99^{**}	-0.99^{**}	-0.24^{ns}
RMR	0.53^{*}	0.54^{*}	0.72^{*}	0.52^{*}	0.73^{*}	0.54^{*}	0.75^{**}	0.12^{ns}	0.34^{ns}	0.20^{ns}	0.88^{**}	0.63^{*}	0.99^{**}	**1**	-1.00^{**}	-0.25^{ns}
SMR	-0.53^{*}	-0.54^{*}	-0.72^{*}	-0.52^{*}	-0.73^{*}	-0.54^{*}	-0.75^{**}	-0.12^{ns}	-0.34^{ns}	-0.20^{ns}	-0.88^{**}	-0.63^{*}	-0.99^{**}	0.23^{ns}	**1**	0.25^{ns}
RTD	0.34^{ns}	0.41^{ns}	0.51^{*}	0.26^{ns}	0.73^{*}	0.27^{ns}	0.52^{*}	-0.01^{ns}	0.26^{ns}	0.10^{ns}	0.89^{**}	0.78^{**}	0.88^{**}	0.86^{**}	-0.86^{**}	1

RSW; root system width (cm), **RSD**; root system depth, **CHA**; convex hull area, **TRL**; total root length, **RBC**; root branch count, **TRV**; total root volume, **FSRL**; first seminal root length, **SRA**; seminal root angle **LA**; leaf area, **LN**; leaf number, **RDM**; root dry mass, **SDM**; shoot dry mass, **RSM**; root–shoot mass ratio, **RMR**; root mass ratio, **SMR**; shoot mass ratio, **RTD**; root tissue density, [***** and ****** denote significant at 5% and 1% probability levels, respectively. **ns**, non-significant]

Table 5.8: Factor loadings, eigenvalue, Kaiser-Meyer-Olkin measure of sampling adequacy, percent and cumulative variation for root and leaf traits of nine citron watermelon accessions evaluated under non-stress and water-stress conditions

	Non-stress			**Water-stress**		
Traits	**PC 1**	**PC 2**	**KMO**	**PC 1**	**PC 2**	**KMO**
RSW	0.792	0.283	0.725	0.898	-0.184	0.870
RSD	0.842	0.429	0.715	0.979	0.049	0.557
CHA	0.927	0.347	0.699	0.968	-0.082	0.575
TRL	0.735	0.570	0.621	0.991	-0.007	0.551
RBC	0.883	-0.121	0.757	0.946	0.184	0.658
TRV	0.802	0.384	0.720	0.906	-0.255	0.633
FSRL	0.798	0.084	0.667	0.888	0.182	0.753
SRA	0.278	0.438	0.499	-0.011	-0.682	0.243
LA	0.548	0.602	0.623	0.872	-0.040	0.530
LN	0.738	-0.416	0.621	0.008	0.936	0.316
RDM	0.942	-0.267	0.746	0.622	0.762	0.441
SDM	0.824	-0.187	0.633	-0.270	0.874	0.542
RSM	0.897	-0.351	0.630	0.955	0.085	0.676
RMR	0.885	-0.338	0.645	0.966	0.066	0.551
SMR	-0.885	0.338	0.645	-0.965	-0.063	0.556
RTD	0.761	-0.553	0.823	-0.369	0.882	0.453
Eigenvalue	10.233	2.369	-	10.319	3.656	-
Variability (%)	63.953	14.807	-	64.497	22.848	-
Cumulative (%)	63.953	78.760	-	64.497	87.345	-

RSW; root system width, **RSD**; root system depth, **CHA**; convex hull area, **TRL**; total root length, **RBC**; root branch count, **TRV**; total root volume, **FSRL**; first seminal root length, **SRA**; seminal root angle **LA**; leaf area, **LN**; leaf number **RDM**; root dry mass, **SDM**; shoot dry mass, **RSM**; root–shoot mass ratio, **RMR**; root mass ratio, **SMR**; shoot mass ratio, **RTD**; root tissue density

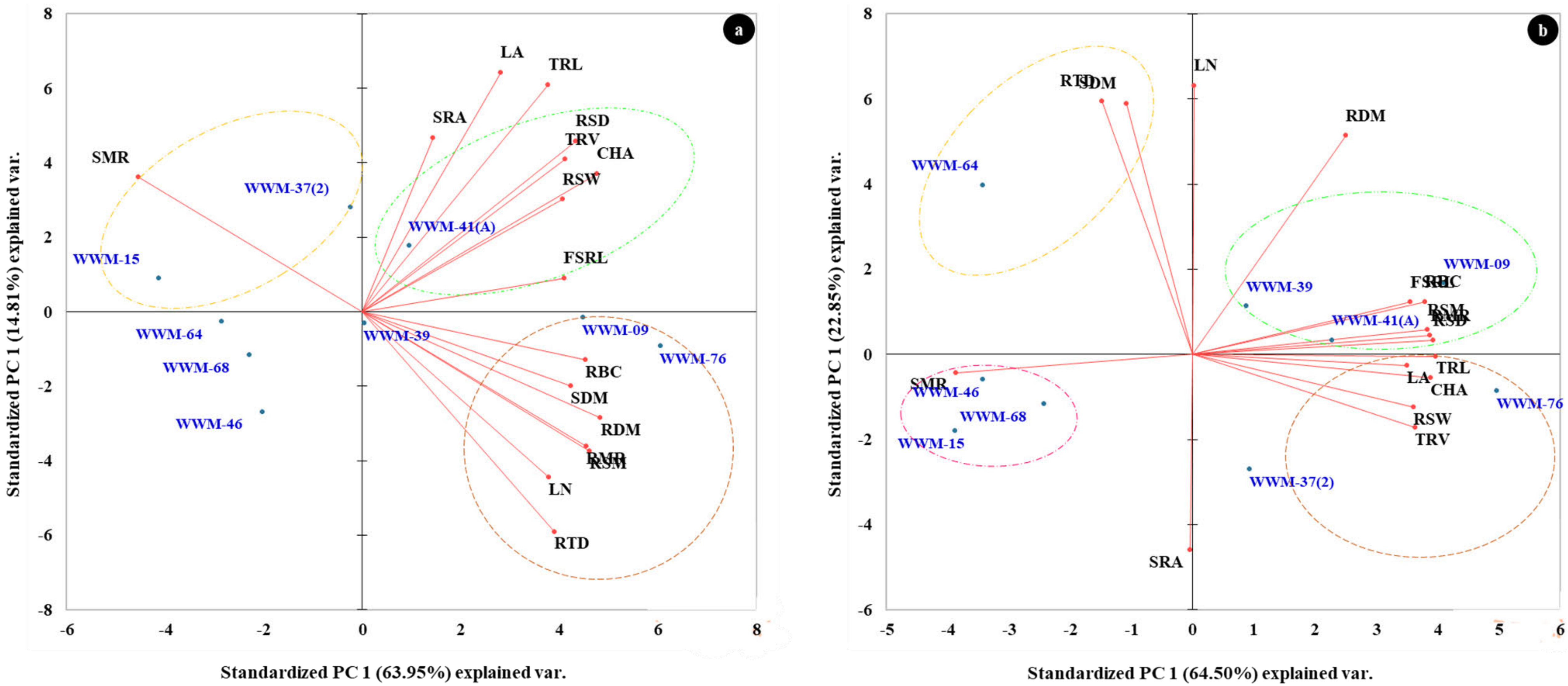

Figure 5.7: Principal component (PC) biplot of PC 1 vs PC 2 demonstrating the relationship between root and leaf traits of 9 citron watermelon accessions evaluated in rhizotrons under (**a**) non-stress and (**b**) water stress conditions

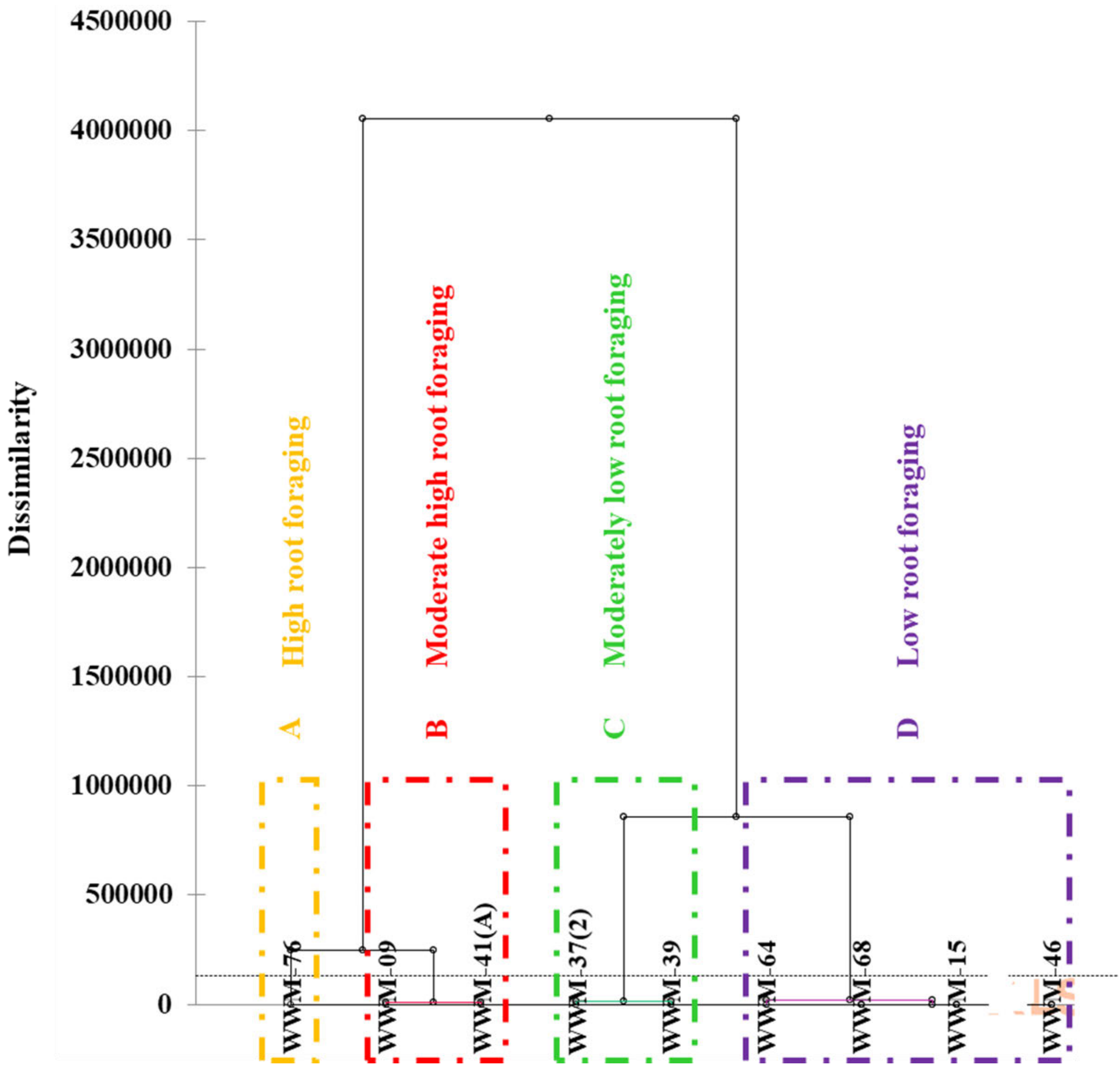

Figure 5.8: Dendrogram distinguishing the levels of root foraging among nine citron watermelon accessions based on measured root system architecture traits under water stress condition

5.4 Discussion

The present study determined the root system architecture of citron watermelon accessions to aid in the selection of key drought-adaptive root traits for breeding targeting water-stressed environments. Root system architecture plays an important role in citron watermelon's response to water stress (Katuuramu et al., 2020). The roots are the first plant organs to respond to water stress. In the present study, the variation in RSA traits among citron watermelon accessions under non-stressed (Table 5.5) and water-stressed conditions (Table 5.6) indicates substantial genetic variability for efficient selection of root-adaptive traits to drought stress. Some important RSA traits for enhanced water-uptake include root system length and width, convex hull area, root branch count and total root length (Katuuramu et al., 2020). In the present study, citron watermelon accessions such as WWM-37(2), WWM-41(A) and WWM-76 increased total root length, convex hull area, root system width and total root volume indicating their ability to absorb water under water stress condition. This agreed with Katuuramu et al. (2020) results that total root length, average root diameter, total root surface area, and total root volume are important RSA traits for adaptation to drought stress in *C. lanatus*, including sweet and citron watermelons.

On the contrary, according to our results, not all accessions evaluated in the present study had increased root length under water stress (Table 5.6). This contradicts the widely generalised view that total root length increases in drier environments (Cavalcante et al., 2019; Narayanan et al., 2014; Qin and Leskovar, 2020; Tomlinson et al., 2012). On contrary, Schenk and Jackson (2002) highlighted that, water availability is not the only abiotic influencing rooting depth, soil texture and genotype composition will also dictate the total root length. The root system architecture is a function of both genetic endogenous programs (regulating growth and organogenesis) and the action of edaphic environmental stimuli. This is supported by a significant interaction between accessions and water conditions.

The efficient response of the root system of the evaluated accessions is also supported by their higher leaf number and shoot biomass compared to other tested accessions. The present study agrees with Guzzon et al. (2017) that citron watermelon exhibit higher above-and-below ground biomass under water deficit conditions as a drought-avoidance strategy. Therefore, the identified RSA traits are recommended for selecting and highly breeding drought-tolerant citron watermelon cultivars in the stir of increased weather conditions in the future. Also, the present findings suggest that citron watermelon can be a donor of root traits for introgression in close related cucurbit species including sweet dessert watermelon to improve drought tolerance and adaption in water-limited environments.

The shift in root growth and allometry observed in the present study can be explained by the "balanced growth" hypothesis, which states that, plants respond to drought by promoting or maintaining root growth while reducing shoot growth (Akmal and Hirasawa, 2004; Glynn et al., 2007; Shipley and Meziane, 2002). Increased root versus shoot growth improved citron watermelon hydraulic status under water stress conditions, probably due to (i) increased root to leaf surface, (ii) continued production of new root tips and (iii) enhancement of plant capacity for acquiring water to support the development of existing shoots. High root: shoot ratio is important; a greater root/shoot ratio means greater root density and root interception for water uptake (Maskova and Herben, 2018). Variation of root: shoot ratio has been reported previously in citron watermelon, whereby drought tolerant citron watermelon genotypes show higher values (Guzzon et al., 2017). Like present finding, citron watermelon accessions WWM-09, WWM-39 and WWM-41(A) had higher root: shoot ratios indicating their higher levels of drought tolerance.

Mandizvo et al. (2022b) highlighted that, as the soil water starts depleting, prolific and deep root systems accompanied with maintenance of leaf surface area is a key attribute of drought-tolerance in citron watermelon. This is supported by positive correlation between root tissue density with shoot biomass (r = 0.84) and leaf number (r = 0.79) (Table 5.7). These observations agree with present findings, indicating that citron watermelon develops a deep root system to allow deep water access and produce high biomass under water-constrained environments. As evidenced by negative associations formed in PC biplots (Figure 5.7b) between SMR with TRL, CHA, RSD and RSW; drought stress induced a conservative balance between water-losing organs (leaves) and water-gaining organs (roots) in the evaluated citron watermelon accessions.

Some RSA traits including deep root system of citron watermelon are preferred rootstock for improving fruit and quality of grafted sweet watermelon for dry water-limited environments (Seymen et al., 2021; Thies et al., 2015; Yavuz et al., 2020). Understanding the interrelationships among below ground (root) growth, above ground (shoot) growth and allometry can provide useful information for an integrated drought tolerance breeding approach. The positive associations observed between root–shoot mass ratio and various root traits under water stress condition including root system width, root system depth, total root length, convex hull area and total root volume suggested synchronised selection and improvement of these traits in citron watermelon.

5.5 Conclusion

The present study compares root system architecture of drought-tolerant citron watermelon accessions to aid the efficient drought-adaptive root traits for cultivar development in water-stressed environments. Using water as a limiting edaphic factor, this study has shown that plasticity and biomass allocation shift in different ways according to genotype, presumably to optimise the use of limited resources. The study found significant phenotypic variation in root architecture among citron watermelon accessions that may relate to differences in water uptake. The following RSA traits including total root length, root system width, convex hull area and total root volume were associated with drought tolerance. Further, RSA traits such as root dry mass and root shoot mass ratio were highly correlated with root branch count, root system depth, total root length and leaf number. These traits are useful selection criteria for breeding and developing water-efficient citron watermelon accessions for cultivation in drought-prone environments.

References

Akmal M., Hirasawa T. (2004) Growth responses of seminal roots of wheat seedlings to a reduction in the water potential of vermiculite. Plant and Soil 267:319-328. DOI: 10.1007/s11104-005-0138-x.

Cavalcante V.S., Prado R.d.M., Vasconcelos R.d.L., Júnior de Almeida H., Ramos da Silva T. (2019) Growth and Nutritional Efficiency of Watermelon Plants Grown under Macronutrient Deficiencies. HortScience 54:738-742. DOI: 10.21273/hortsci13807-18.

Chomicki G., Renner S.S. (2015) Watermelon origin solved with molecular phylogenetics including Linnaean material: another example of museomics. New Phytol 205:526-32. DOI: 10.1111/nph.13163.

Crosby K.M., Jifon J.L., Leskovar D.I. (2008) Genetic Improvement of Early Root Vigor in Melon (*Cucumis melo* L.) to Enhance Stand Establishment. Acta Horticulturae:273-278. DOI: 10.17660/ActaHortic.2008.782.33.

de Kroon H., Huber H., Stuefer J.F., van Groenendael J.M. (2005) A modular concept of phenotypic plasticity in plants. New Phytol 166:73-82. DOI: 10.1111/j.1469-8137.2004.01310.x.

Easlon H.M., Bloom A.J. (2014) Easy Leaf Area: Automated digital image analysis for rapid and accurate measurement of leaf area. Appl Plant Sci 2. DOI: 10.3732/apps.1400033.

Fita A., Picó B., Nuez F. (2007) Melon roots under stress: melon vine decline. Plant Stress 1:104.

Gloaguen R.M., Brym Z.T., Peeples J., Xu W., Chun H.-C., Rowland D.L. (2022) The plasticity of early root development in *Sesamum indicum* L. as influenced by genotype, water, and nutrient availability. Rhizosphere 21. DOI: 10.1016/j.rhisph.2021.100457.

Glynn C., Herms D.A., Orians C.M., Hansen R.C., Larsson S. (2007) Testing the growth-differentiation balance hypothesis: dynamic responses of willows to nutrient availability. New Phytol 176:623-634. DOI: 10.1111/j.1469-8137.2007.02203.x.

Guzzon F., Müller J.V., do Nascimento Araujo M., Cauzzi P., Orsenigo S., Mondoni A., Abeli T. (2017) Drought avoidance adaptive traits in seed germination and seedling growth of *Citrullus amarus* landraces. South African Journal of Botany 113:382-388. DOI: 10.1016/j.sajb.2017.09.023.

Haney R.L., Haney E.B. (2010) Simple and Rapid Laboratory Method for Rewetting Dry Soil for Incubations. Communications in Soil Science and Plant Analysis 41:1493-1501. DOI: 10.1080/00103624.2010.482171.

Hong T., Cai Z., Li R., Liu J., Li J., Wang Z., Zhang Z. (2022) Effects of water and nitrogen coupling on watermelon growth, photosynthesis and yield under CO_2 enrichment. Agricultural Water Management 259. DOI: 10.1016/j.agwat.2021.107229.

Katuuramu D., Branham S., Levi A., Wechter P. (2021) Genome-wide association analysis of resistance to *Pseudoperonospora cubensis* in citron watermelon. Plant Dis. DOI: 10.1094/PDIS-08-21-1611-RE.

Katuuramu D.N., Wechter W.P., Washington M.L., Horry M., Cutulle M.A., Jarret R.L., Levi A. (2020) Phenotypic diversity for root traits and identification of superior germplasm for root breeding in watermelon. HortScience 55:1272-1279. DOI: 10.21273/hortsci15093-20.

Koevoets I.T., Venema J.H., Elzenga J.T., Testerink C. (2016) Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. Front Plant Sci 7:1335. DOI: 10.3389/fpls.2016.01335.

Maja D., Mavengahama S., Mashilo J. (2021) Cucurbitacin biosynthesis in cucurbit crops, their pharmaceutical value and agricultural application for management of biotic and abiotic stress: A review. South African Journal of Botany. DOI: 10.1016/j.sajb.2021.08.044.

Mandizvo T., Odindo A.O., Mashilo J. (2021) Citron Watermelon Potential to Improve Crop Diversification and Reduce Negative Impacts of Climate Change. Sustainability 13. DOI: 10.3390/su13042269.

Mandizvo T., Odindo A.O., Mashilo J. (2022a) Nutrient composition and physical properties of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) seeds are related to seed coat visual appearance. South African Journal of Botany. DOI: 10.1016/j.sajb.2022.02.015.

Mandizvo T., Odindo A.O., Mashilo J., Magwaza L.S. (2022b) Drought tolerance assessment of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) accessions based on morphological and physiological traits. Plant Physiology and Biochemistry 180:106-123. DOI: 10.1016/j.plaphy.2022.03.037.

Mashilo J., Shimelis H., Ngwepe R.M. (2021) Genetic resources of bottle gourd (*Lagenaria siceraria* (Molina) Standl.] and citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.): implications for genetic improvement, product development and commercialization: a review. South African Journal of Botany. DOI: 10.1016/j.sajb.2021.10.013.

Mashilo J., Shimelis H., Odindo A., Amelework B. (2016) Simple Sequence Repeat Markers Reveal Genetic Diversity Within and among Landrace Collections of Citron and Dessert Watermelon from South Africa. Journal of the American Society for Horticultural Science 141:598-608. DOI: 10.21273/jashs03870-16.

Mashilo J., Shimelis H., Odindo A., Amelework B. (2017a) Assessment of the genetic diversity of dessert watermelon (*Citrullus lanatus* var. *lanatus*) landrace collections of South Africa using SSR markers. Australian Journal of Crop Science 11:1392-1398. DOI: 10.21475/ajcs.17.11.11.pne461.

Mashilo J., Shimelis H., Odindo A.O., Amelework B. (2017b) Genetic diversity and differentiation in citron watermelon [*Citrullus lanatus* var. *citroides*] landraces assessed by simple sequence repeat markers. Scientia Horticulturae 214:99-106. DOI: 10.1016/j.scienta.2016.11.015.

Maskova T., Herben T. (2018) Root:shoot ratio in developing seedlings: How seedlings change their allocation in response to seed mass and ambient nutrient supply. Ecol Evol 8:7143-7150. DOI: 10.1002/ece3.4238.

Narayanan S., Mohan A., Gill K.S., Prasad P.V. (2014) Variability of root traits in spring wheat germplasm. PLoS One 9:e100317. DOI: 10.1371/journal.pone.0100317.

Ngwepe R.M., Mashilo J., Shimelis H. (2019) Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides*): a review. Genetic Resources and Crop Evolution 66:735-758. DOI: 10.1007/s10722-018-0724-4.

Nkoana D.K., Mashilo J., Shimelis H., Ngwepe R.M. (2021) Nutritional, phytochemical compositions and natural therapeutic values of citron watermelon (*Citrullus lanatus* var. *citroides*): A Review. South African Journal of Botany. DOI: 10.1016/j.sajb.2020.12.008.

Omirou M., Ioannides I.M., Ehaliotis C. (2013) Mycorrhizal inoculation affects arbuscular mycorrhizal diversity in watermelon roots, but leads to improved colonization and plant response under water stress only. Applied Soil Ecology 63:112-119. DOI: 10.1016/j.apsoil.2012.09.013.

Qin K., Leskovar D.I. (2020) Humic Substances Improve Vegetable Seedling Quality and Post-Transplant Yield Performance under Stress Conditions. Agriculture 10. DOI: 10.3390/agriculture10070254.

Rentería-Martínez M.E., Guerra-Camacho M.Á., Ochoa-Meza A., Moreno-Salazar S.F., Varela-Romero A., Gutiérrez-Millán L.E., Meza-Moller A.D.C. (2018) Análisis filogenético multilocus del complejo fúngico asociado a pudrición radicular de sandía en Sonora, México. Revista Mexicana de Fitopatología, Mexican Journal of Phytopathology 36. DOI: 10.18781/r.Mex.Fit.1710-1.

Roy A., Bal S.S., Fergany M., Kaur S., Singh H., Malik A.A., Singh J., Monforte A.J., Dhillon N.P.S. (2012) Wild melon diversity in India (Punjab State). Genetic Resources and Crop Evolution 59:755-767. DOI: 10.1007/s10722-011-9716-3.

Schenk H.J., Jackson R.B. (2002) Rooting Depths, Lateral Root Spreads and Below-Ground/Above-Ground Allometries of Plants in Water-Limited Ecosystems. Journal of Ecology 90:480-494.

Schwarz D., Rouphael Y., Colla G., Venema J.H. (2010) Grafting as a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. Scientia Horticulturae 127:162-171. DOI: 10.1016/j.scienta.2010.09.016.

Seymen M., Yavuz D., Ercan M., Akbulut M., Çoklar H., Kurtar E.S., Yavuz N., Süheri S., Türkmen Ö. (2021) Effect of wild watermelon rootstocks and water stress on chemical properties of watermelon fruit. Horticulture, Environment, and Biotechnology 62:411-422. DOI: 10.1007/s13580-020-00329-4.

Shipley B., Meziane D. (2002) The balanced-growth hypothesis and the allometry of leaf and root biomass allocation. Functional Ecology 16:326-331. DOI: 10.1046/j.1365-2435.2002.00626.x.

Thies J.A., Ariss J.J., Hassell R.L., Buckner S., Levi A. (2015) Accessions of *Citrullus lanatus* var. *citroide*s are valuable rootstocks for grafted watermelon in fields infested with root-knot nematodes. HortScience 50:4-8. DOI: 10.21273/hortsci.50.1.4.

Thies J.A., Levi A. (2007) Characterization of Watermelon (*Citrullus lanatus* var. *citroides*) Germplasm for Resistance to Root-knot Nematodes. HortScience 42:1530-1533. DOI: 10.21273/hortsci.42.7.1530.

Tomlinson K.W., Sterck F.J., Bongers F., da Silva D.A., Barbosa E.R.M., Ward D., Bakker F.T., van Kaauwen M., Prins H.H.T., de Bie S., van Langevelde F. (2012) Biomass partitioning and root morphology of savanna trees across a water gradient. Journal of Ecology 100:1113-1121. DOI: 10.1111/j.1365-2745.2012.01975.x.

Uygur V., Yetisir H. (2009) Effects of Rootstocks on Some Growth Parameters, Phosphorous and Nitrogen Uptake Watermelon under Salt Stress. Journal of Plant Nutrition 32:629-643. DOI: 10.1080/01904160802715448.

Wiese A.H., Riemenschneider D.E., Ronald Jr S. (2005) An inexpensive rhizotron design for two-dimensional, horizontal root growth measurements. Tree Planters' Notes. 51: 40-46. 51.

Yavuz D., Seymen M., Süheri S., Yavuz N., Türkmen Ö., Kurtar E.S. (2020) How do rootstocks of citron watermelon (*Citrullus lanatus* var. *citroides*) affect the yield and quality of watermelon under deficit irrigation? Agricultural Water Management 241. DOI: 10.1016/j.agwat.2020.106351.

Chapter 6: Phytosterols augment endurance against interactive effects of heat and drought stress on biochemical activities of *Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. Ex Greb)

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Abstract

Water deficit and heat have been considered the main abiotic stresses to which plants are exposed. Since seedling establishment is a vulnerable developmental transition that plays a critical role in crop production, we carried out an *in-vitro* experiments to reveal how seedlings of the citron watermelon respond to water deficit and heat with respect to growth, water status, reserve mobilisation, hydrolase activity and metabolite partitioning, including non-structural carbohydrate availability. To reveal the involvement of phytosterols (stigmasterol, sitosterol and campesterol) in combined stress tolerance, four citron watermelon genotypes were investigated under varying osmotic potential [-0.05 MPa, -0.09 MPa and -0.19MPa] and temperature (26 °C and 38°C). Phytosterols were analyzed by gas chromatography−mass spectrometry (GC−MS). In the seedlings exposed to osmotic stress induced by polyethylene glycol (PEG), restricted growth was accompanied by alterations in relative water content (RWC) and associated with delayed mobilisation of starch in the cotyledons and decreased availability of non-structural carbohydrates in the root. By contrast, high temperature retarded the photosynthetic apparatus's establishment and compromised photosynthetic pigments activity and dry matter production. Our results suggest that inborn stress tolerance in citron watermelon is manifested by enhanced accumulation of most lipids, mainly sterols, especially in heat/drought-stressed plants. This study provides valuable information about the metabolic response of citron watermelon to combined stress and metabolites identified, which will encourage further study by transcriptome and proteomics to improve drought tolerance in citron watermelon.

Keywords: Campesterol, Stigmasterol, Sitosterol, Combined stress

6.1 Introduction

Essential features of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. Ex Greb.) contribute to its broad use in genetic studies (Mashilo et al., 2021). These traits include the crop's diploid nature with a high degree of inbreeding, its low chromosome number, and ease of cross-breeding and cultivation in various climatic conditions (Mashilo et al., 2017; Ngwepe et al., 2019). Due to its geographic adaptability and natural tolerance to drought, heat, or salinity, there is an increasing interest in identifying the stress response mechanisms in citron watermelon.

Numerous studies have focused on its response to abiotic stresses, such as drought (Mandizvo et al., 2022; Yavuz et al., 2020), heat (Saroj and Choudhary, 2020) and salinity (Gomes do Ó et al., 2022; Santos et al., 2022; Yavuz, 2021). Most of these reports centered on the influence of single stress; however, plants are usually exposed to multiple abiotic stresses under field conditions. For example, drought is often accompanied by high temperatures, and their combined effects significantly impact citron watermelon yield more than the effects of a single stress alone.

Reports have revealed that the reaction of plant(s) to combined stresses is unique and cannot be directly extrapolated from the response of the plant exposed to individual stress (Demirel et al., 2020; Magana Ugarte et al., 2019; Mittler, 2006; Zandalinas et al., 2022). However, it is known that drought (Akashi et al., 2001; Ansari et al., 2019), high temperature (Rivero et al., 2001; Yonny et al., 2016) and salinity (Huang et al., 2010; Zhao et al., 2020) induce oxidative damage, and thus similar molecular responses may also occur in plants.

The available data on the combined effects of abiotic stresses on citron watermelon is limited, especially regarding changes in lipids under multiple abiotic stresses (Mandizvo et al., 2021). Improved high resolution and sensitivity in mass spectrometry (MS) have facilitated the identification and characterization of key compounds in biological processes, including metabolites, proteins, and lipids (Feng et al., 2022; Li et al., 2022; Schlag et al., 2022; Shuai et al., 2022). Due to technological advances, modern lipidomic approaches use optimized and tailored MS-based methods (Rupasinghe and Roessner, 2018) and these have been successfully applied to plant lipid research (Engel et al., 2022).

Lipids (a group of biomolecules) are present in all plant tissues; they exert multiple roles and functions: constituents of the cell membrane (Mamode Cassim et al., 2019), storage molecules of metabolic energy (Correa et al., 2020), and as signaling factors in response to stressors (Hou et al., 2016). Considering the different lipid classes, sterols are of great importance as they exert a structural function in cell membranes, contributing to modulation of their fluidity. Sterols exist in a free form or as esterified molecules with fatty acids or carbohydrates. Phytosterols mainly comprise of campesterol, β-sitosterol and stigmasterol (Rogowska and Szakiel, 2020; Valitova et al., 2016).

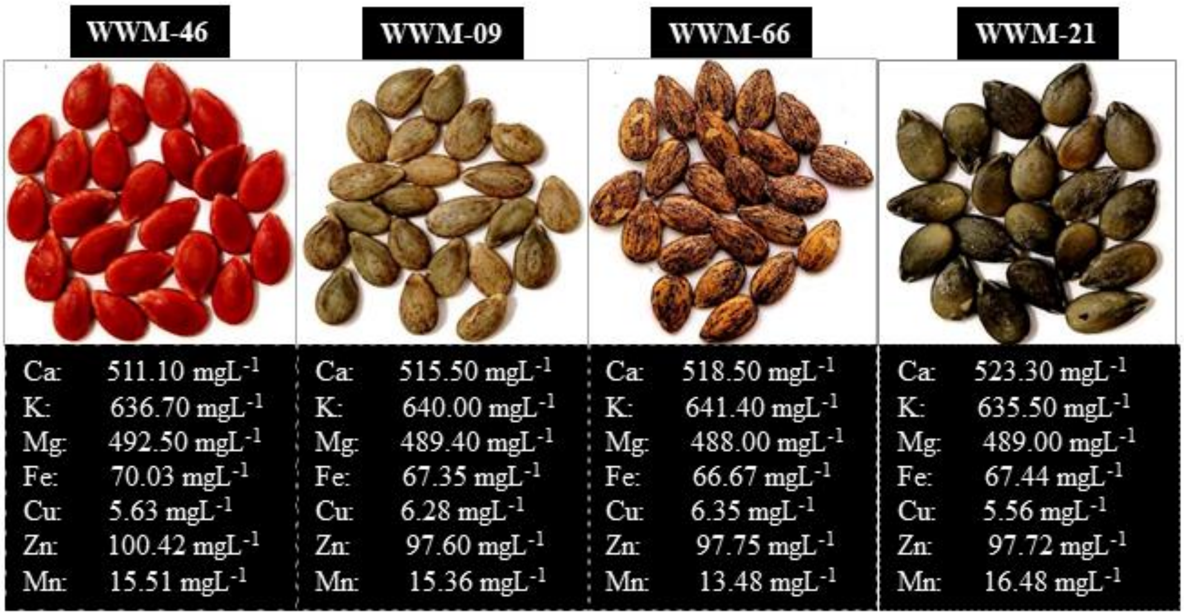
Apart from their structural function, phytosterols also play a regulatory role in plants. The relative phytosterol composition is altered by stress, changing the characteristics of the cell membrane and its biological functions (Roche et al., 2008). Moreover, it is assumed that plant adaptation to stress may be determined by the ability of phytosterols to resist oxidation by reactive oxygen species (ROS) that are generated under various stress conditions. Reactive oxygen species react with unsaturated molecules, changing their structure and cellular functions (Hossain et al., 2016). Dufourc (2008) reported that campesterol is a precursor for biosynthesis of brassinosteroids, a key group of steroidal hormones involved in plant growth and development. All these facts justify sterols' fundamental role in stress response.

We hypothesized that phytosterols changes in citron watermelon seedling axis are differentially expressed under combined stress compared to a single stress. Second, we postulated that genetics and environmental factors interact to influence phytosterol changes, and this study demonstrated the significance of both factors. Therefore, the objective of this study was to identify the multiple abiotic stress-induced modifications in different phytosterols (campesterol, sitosterol and stigmasterol) in seedling axis (embryonic leaf and root) of genetically distinct citron watermelon accessions. Detailed evaluation of phytosterols was done and the effects of the changes observed in stressed plants were discussed.

6.2 Materials and methods

6.2.1 Plant material

The Limpopo Department of Agriculture and Rural Development; South Africa provided forty citron watermelon landrace accessions. Four genotypes were selected from previous work based on high-stress tolerance index (Mandizvo et al., 2022). The mineral element composition of selected genotypes are summarised in (Figure 6.1).



	WWM-46	WWM-09	WWM-66	WWM-21
Ca:	511.10 mgL^{-1}	515.50 mgL^{-1}	518.50 mgL^{-1}	523.30 mgL^{-1}
K:	636.70 mgL^{-1}	640.00 mgL^{-1}	641.40 mgL^{-1}	635.50 mgL^{-1}
Mg:	492.50 mgL^{-1}	489.40 mgL^{-1}	488.00 mgL^{-1}	489.00 mgL^{-1}
Fe:	70.03 mgL^{-1}	67.35 mgL^{-1}	66.67 mgL^{-1}	67.44 mgL^{-1}
Cu:	5.63 mgL^{-1}	6.28 mgL^{-1}	6.35 mgL^{-1}	5.56 mgL^{-1}
Zn:	100.42 mgL^{-1}	97.60 mgL^{-1}	97.75 mgL^{-1}	97.72 mgL^{-1}
Mn:	15.51 mgL^{-1}	15.36 mgL^{-1}	13.48 mgL^{-1}	16.48 mgL^{-1}

Figure 6.1: Citron watermelon genotypes used for the study and their estimated mineral element composition from fast atomic absorption spectrometry (FAAS)

6.2.2 In-vitro culture

6.2.2.1 Water agar preparation

Twenty grams of agar powder for tissue culture [CAS No. 9002-18-0] purchased from Sisco Research Laboratories, India, was measured using an analytical balance (Shimadzu AP124W) and suspended in 1000 ml double distilled water. The agar was boiled to dissolve it completely. Completely dissolved water agar was sterilised by autoclaving at 15 Pa (121 °C) for 15 minutes in a Biobase autoclave (Model: BKQ-B50II).

6.2.2.2 Design of simulated water stress conditions

Following the method described by Guo et al. (2012), 0, 5 and 10% polyethylene glycol (PEG) solutions with osmotic potentials of 0.00, -0.09 and -0.19 MPa, respectively, were prepared. The solutions' osmotic potential (OP) in Table 6.1 was measured using a CX-2 water potential meter (Decagon Devices, Inc. Pullman, Washington).

Table 6.1: Osmotic potential of polyethylene glycol (PEG-6000) solutions

PEG-6000 concentration (%)	**Osmotic potential (MPa)**
0 (control)	-0.05
5	-0.09
10	-0.19

6.2.3 Water and heat stress treatments

The experiment was a 4 (genotypes) × 3 (osmotic potential) × 2 (temperature regimes) factorial design replicated three times to give 72 experimental units. Day-old seedlings were transferred to 100 ml transparent cups containing 5 gL^{-1} water agar for water stress treatment. Drought stress was induced by injecting 15 ml of PEG solution of different OPs (0.00, -0.09 and -0.19 MPa) into the water agar using a syringe. Lower OPs were avoided as they cease seedling development. The top of the agar was covered with cotton wool to reduce agar contamination. Transparent cups were covered with aluminium foil paper to block light from influencing root growth. The cups were placed in a growth chamber (Micro-Clima Arabidopsis Chamber, ECP01E, Snijders, Netherlands) for 5 days. Growth chamber conditions were set at 25 ± 1 °C, 70% relative humidity, illumination of 4000 lux for 12 hours and 350 ppm CO_2. Set values were controlled by the control unit (JUMO IMAGO 500). Day-old seedlings were transplanted to 5 g L^{-1} water agar in 100 ml transparent cups for heat stress treatment and maintained in an incubator at 26 °C (control) and 38 °C (heat stress) for 5 days.

6.2.4 *Seedling growth*

The average daily growth rate (ADGR) for the seedling under different treatments was measured according to Equation 6.1, where H_1 and H_2 are plant height at times T_1 and T_2.

$$\mathbf{ADGR} = \left[\frac{H_2 - H_1}{T_2 - T_1}\right]$$

(Equation 6.1) (Kubota, 2016)

Five days after taking daily growth measurements, seedlings were uprooted, washed and sectioned into cotyledon, hypocotyl and roots. Fresh mass was measured soon after uprooting and dry mass was measured after samples were oven-dried for 48 hours at 75 °C.

6.2.5 *Relative water content (RWC)*

Samples of the different seedling parts whose fresh weight (FW) was previously measured were immersed in distilled H_2O and maintained at 25 °C for 60 minutes. The samples were blotted on paper towels to remove excess water and weighed to quantify the turgid weight (TW) using an analytical balance (Shimadzu AP124W). Finally, the samples were oven-dried at 75 °C for 48 hours to obtain the DW. Equation 6.2 was used to calculate the relative water content (RWC).

$$\mathbf{RWC} = \left[\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\right] \times 100$$

(Equation 6.2) (Qin et al., 2019)

6.2.6 *Biochemical analysis samples*

Samples reserved for the biochemical analysis were frozen at -70 °C in a freezer (HERAfreeze TDE TDE50086FV) for 24 hours and lyophilised in a freeze drier (Larry Virtis 255L) at -126.5 ºC for 96 hours. Dried embryonic leaves, hypocotyl and roots were separately ground into a fine powder using mortar and pestle and stored in a chest freezer at -5 ºC.

6.2.7 Estimation of photosynthesis pigments

Total chlorophyll content, chlorophyll '*a*', chlorophyll '*b*' and carotenoids were extracted and quantified using the method described by (Meher et al., 2018). Total chlorophylls and carotenoids were extracted from 50 mg of fresh leaf tissue by maceration with 10 ml of 80% (v/v) acetone (C_3H_6O) under reduced luminosity. Samples were centrifuged at 6000 rpm for 20 minutes using a GenFuge 24D (Mexborough, England). The supernatants were collected, and readings were taken at 450, 645 and 663 nm using Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer. Following the extraction and analysis, the relative amounts of chlorophyll '*a*' chlorophyll '*b*' and total chlorophyll content were estimated using Equation 6.3, 6.4 and 6.5.

$$\textbf{Chlorophyll}\ 'a' = \frac{(12.7 \times A_{663} - 2.69 \times A_{645}) \times V}{W} \qquad \text{(Equation 6.3)}$$

$$\textbf{Chlorophyll}\ 'b' = \frac{(22.9 \times A_{645} - 4.68 \times A_{663}) \times V}{\mathrm{W}} \qquad \text{(Equation 6.4)}$$

$$\textbf{Total chlorophyll} = \frac{(20.2 \times A_{645} + 8.02 \times A_{663}) \times V}{W} \qquad \text{(Equation 6.5)}$$

A; Absorbance at specific wavelengths, ***V***; final volume of chlorophyll extract in 80% acetone, ***W***; fresh weight of tissue extracted, **Constants**; 12.7, 2.69, 22.9, 4.68, 20.2 and 8.02

6.2.8 Non-structural carbohydrates (NSC)

Soluble metabolites were extracted from 200 mg of frozen FW, which were fragmented and transferred to tubes containing 1.5 mL of 80% (v/v) ethanol. The tubes were sealed with parafilm tape and incubated at 60 ºC for 30 minutes. Supernatants were harvested, and the residues were extracted again to yield 3 ml of ethanolic extract per sample. Total soluble sugars (TSS) were quantified with the anthrone reagent, using D-glucose as standard (Turakainen et al., 2004). Non-reducing sugars (NRS) were measured by modifying the anthrone method, employing a sucrose standard curve (Panjekobi and Einali, 2021). The content of both metabolites (TSS and NRS) were calculated as µmol g^{-1} DW.

Starch was extracted from pellets obtained after the extraction of soluble metabolites. The pellets were macerated with 0.5 ml of 30% (v/v) perchloric acid ($HClO_4$) and the homogenates were centrifuged at 6000 rpm for 20 minutes. The supernatants were collected, and the pellets were re-extracted twice, yielding 1.5 ml of extract per sample. These procedures were performed at ≈ 4 °C. Starch was also determined using a D-glucose standard curve with the anthrone method (Maharjan et al., 2018). The starch content was calculated according to (McCready et al., 2002) and expressed as mg $part^{-1}$.

6.2.9 *Oxidative stress marker (malondialdehyde)*

Following the method described by Hodges et al. (1999), malondialdehyde (MDA) was measured in 80% (v/v) methanol extracts of 100 mg dry plant material. Extracts were mixed with 0.5% thiobarbituric acid ($C_4H_4N_2O_2S$) prepared in 20% trichloroacetic acid (TCA), (or with 20% TCA without TBA for the controls), and then incubated at 95 °C for 20 minutes. After stopping the reaction, the supernatant absorbance was measured at 532 nm. The non-specific absorbance at 600 and 440 nm was subtracted and MDA concentration was calculated using the equations described by (Cicevan et al., 2016; Hodges et al., 1999).

$$\boldsymbol{A} = [(Abs532 + TBA) - (Abs600 + TBA) - (Abs532 - TBA - Abs600 - TBA)]$$ (Equation 6.6)

$$\boldsymbol{B} = [(Abs\ 440 + TBA - Abs\ 600 + TBA)\ 0.0571]$$ (Equation 6.7)

$$\boldsymbol{MDA\ equivalents} = [\frac{A-B}{157000}] \times 10^6$$ (Equation 6.8)

6.2.10 *Phytosterols analysis*

Phytosterols (campesterol, stigmasterol and sitosterol) were estimated following an analytical method described by (Tan et al., 2019). Two grams of freeze-dried samples (cotyledons, hypocotyl and roots) were dissolved in 10 ml *n*-Hexane (chromatographic reagent grade, purchased from Chem Lab Supplies (Benrose, Johannesburg)) for 30 minutes. The mixture was filtered through Whatman No. 5 filter paper, and the aliquot of samples filtrate was stored in scintillation vials. Analysis was done using a GCMS-QP2010 SE (Shimadzu, Japan) equipped with a ZB-5MSplus column (30 m x 0.25 mm i.d. × 0.25µm film thickness) (Merck KGaA, Darmstadt, Germany) and deactivated tubing guard column (Zebron 5m x 0,25mm).

For each sample, 1.0 µl was injected at 310 °C in the split mode (split ratio 20:1). Helium was used as the carrier gas at a flow rate (1.8 ml min^{-1}). The GC temperature program was initiated at 200 °C, held for 30 seconds, increased to 310 °C at 30 °C min^{-1}, and held for 10 minutes. Electron ionisation (EI) source was applied, and electron energy was 70 eV. The source and interface temperatures were set at 230 °C and 310 °C, respectively. The mass analyser was set in the selected ion monitoring mode for qualitative and quantitative analyses. For qualitative analyses, values of the ions selected were *m/z* 382, 147, and 81 to identify campesterol, *m/z* 394, 255, and 81 for stigmasterol, and *m/z* 396, 213, and 43 for sitosterol.

6.2.11 *Statistical analysis*

A combined two-way analysis of variance was performed for measured parameters using Genstat 20th edition (VSN International, Hempstead, United Kingdom). Means were separated using Fisher's protected least significant difference (LSD) test when treatments showed significant effects on measured parameters at 5% level of significance. Principal component analysis (PCA) and the biplot diagrams were exploited to identifying the main axes of variance within a data set using XLSTAT software (Data Analysis and Statistical Solution for Microsoft Excel, Addinsoft, Paris, France, 2022).

6.3 Results

6.3.1 Seedling growth

After five days of treatment exposure, a decrease in plant height was intricately linked with decreasing osmotic potential and increasing temperature (Figure 6.2). The highest plant height (10.267 cm) was observed in WWM-09 at [-0.05 MPa; 26 °C] after five days of treatment exposure (Figure 6.2a). After five days of treatment exposure, the least plant height (3.300 cm) was recorded in WWM-09 at [-0.19 MPa; 38 °C] (Figure 6.2f).

At [-0.05 MPa; 26 °C], plant height after five days ranged from 8.867 in WWM-66 to 10.267 cm in WWM-09 (Figure 6.2a). At [-0.09 MPa; 26 °C], plant height ranged from 6.900 cm in WWM-46 to 7.900 cm in WWM-21 (Figure 6.2b). Highest (5.600 cm) and lowest (4.900 cm) plant height were observed in WWM-21 and WWM-46, respectively, at [-0.19 MPa; 26 °C] (Figure 6.2c).

Landrace accessions WWM-21 and WWM-46 recorded the lowest (5.000 cm) and highest (5.767 cm) plant height at [-0.05 MPa; 38 °C] (Figure 6.2d). At [-0.09 MPa; 38 °C], plant height ranged from 4.400 cm (WWM-66) to 5.000 cm (WWM-46) (Figure 6.2e). At [-0.19 MPa; 38 °C], plant height ranged from 3.300 cm in WWM-09 to 4.600 cm in WWM-46 (Figure 6.2f).

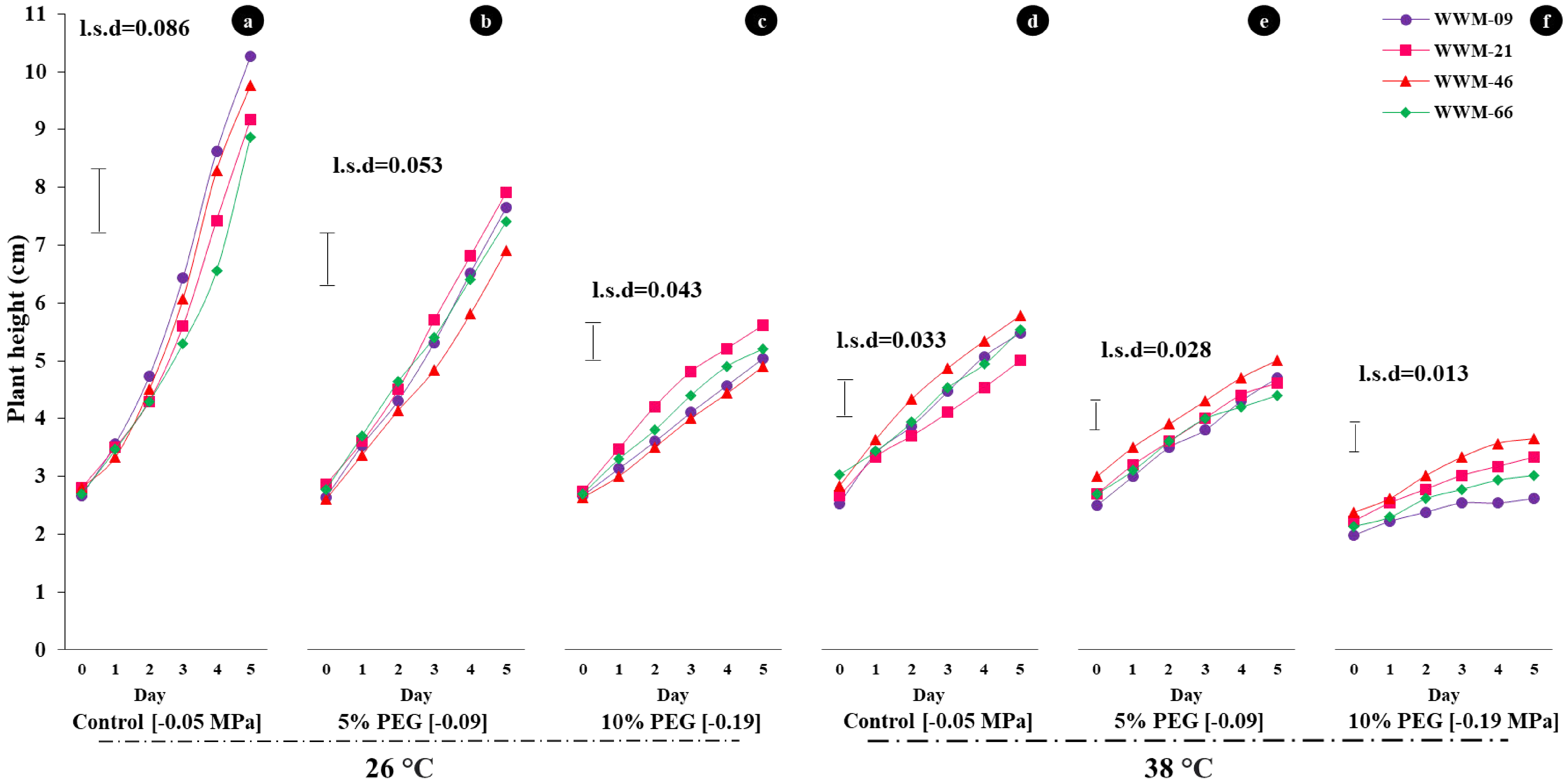


Figure 6.2: Effect of heat and water stress on hypocotyl growth of four citron watermelon landrace accession over five days after exposure to combined stress (water and heat) treatment

There were significant differences ($P < 0.001$) in average daily growth rate (ADGR) of citron watermelon seedlings under varying osmotic potential and temperature. The ADGR values at [-0.05 MPa; 26 °C] ranged from 1.175 cm day^{-1} in WWM-66 to 1.569 cm day^{-1} in WWM-09. The treatment combination [-0.09 MPa; 26 °C] reduced the ADGR to a range of 0.843 to 1.028 cm day^{-1}. At [-0.19 MPa; 26 °C] highest average daily growth rate (0.575 cm day^{-1}) was recorded in WWM-21 and the lowest (0.475 cm day^{-1}) was recorded in WWM-09 (Table 6.2).

Landrace accessions WWM-46 and WWM-21 recorded the highest (0.580 cm day^{-1}) and lowest (0.448 cm day^{-1}) ADGR under [-0.05 MPa; 38 °C]. At [-0.09 MPa; 38 °C], WWM-09 recorded the highest ADGR and WWM-66 recorded the least ADGR of 0.434 cm day^{-1} and 0.349 cm day^{-1}, respectively. At [-0.19 MPa; 38 °C], WWM-46 had highest ADGR (0.343 cm day^{-1}), followed by WWM-21 (0.277 cm day^{-1}), WWM-66 (0.231 cm day^{-1}) and WWM-09 (0.154 cm day^{-1}) (Table 6.2).

Table 6.2: Mathematical representation ($y = mx + c$) of average daily growth rate (cm day-1) of citron watermelon accessions under heat and water stress

	Average daily growth rate (cm day^{-1})					
	Temperature (26 °C)			Temperature (38 °C)		
Genotype	**Control**	**5% PEG**	**10% PEG**	**Control**	**5% PEG**	**10% PEG**
WWM-09	1.569^{a}	0.997^{a}	0.475^{ab}	0.579^{a}	0.434^{a}	0.154^{d}
WWM-21	1.284^{c}	1.028^{a}	0.575^{a}	0.448^{c}	0.386^{c}	0.277^{b}
WWM-46	1.466^{b}	0.843^{b}	0.461^{b}	0.580^{a}	0.400^{b}	0.343^{a}
WWM-66	1.175^{c}	0.997^{a}	0.511^{a}	0.503^{b}	0.349^{d}	0.231^{c}
LSD	0.086	0.053	0.043	0.033	0.028	0.013
CV%	3.300	4.200	3.900	5.900	3.400	4.500
P-value	0.044	<.001	<.001	<.001	<.001	<.001

Means in the same column followed by the same letter are not significantly different, while figures with different superscript letters are significantly different according to Fisher's test. ADGR is the coefficient of x in the linear equation ($y = mx + c$) extracted from graphs in Figure 6.2

6.3.2 *Seedling axis*

Cotyledon dry mass (CDM), hypocotyl dry mass (HDM) and root dry mass (RDM) were significantly different ($P < 0.05$) with significant interactions among landraces at both temperature regimes (26 °C and 38 °C) and varying osmotic potentials (-0.05, -0.09 and -0.19 MPa) (Figure 6.3).

The cotyledon dry mass at [-0.05 MPa; 26 °C] ranged from 0.584 g in WWM-09 to 0.514 g in WWM-16 (Figure 6.3a). Accessions WWM-09 and WWM-66 recorded the highest (0.444 g) and lowest (0.372 g) CDM under [-0.09 MPa; 26 °C], respectively (Figure 6.3b). At [-0.19 MPa; 26 °C], highest CDM (0.267 g) was recorded in WWM-09 and WWM-21, while the least CDM (0.219 g) was recorded in WWM-46 (Figure 6.3c). WWM-09 had the highest CDM of

0.205 g, while WWM-46 recorded the lowest CDM (0.179 g) at [-0.05 MPa; 38 °C] (Figure 6.3d). In Figure 6.3e ([-0.09 MPa; 38 °C]) WWM-09 recorded the highest CDM (0.192 g) and WWM-66 had the lowest CDM (0.148 g). The highest (0.147 g) and the lowest (0.122 g) CDM at [-0.19 MPa; 38 °C] were recorded in WWM-09 and WWM-66, respectively (Figure 6.3f).

Hypocotyl dry mass (HDM) at [-0.05 MPa; 26 °C] ranged from 0.398 g in WWM-21 to 0.445 g in WWM-09 (Figure 6.3g). Accessions WWM-09 and WWM-21 recorded the highest (0.349 g) and lowest (0.299 g) HDM at [-0.09 MPa; 26 °C], respectively (Figure 6.3h). At [-0.19 MPa; 26 °C], highest HDM (0.289 g) was recorded in WWM-09 and the least HDM (0.195 g) was recorded in WWM-21 (Figure 6.3i). WWM-09 had the highest HDM of 0.175 g, while WWM-46 recorded the lowest HDM (0.165 g) at [-0.05 MPa; 38 °C] (Figure 6.3j). The highest (0.180 g) and the lowest (0.171 g) HDM at [-0.09 MPa; 38 °C] were recorded in WWM-21 and WWM-66, respectively (Figure 6.3k). Accessions WWM-46 and WWM-09 recorded higher HDM values (0.122 and 0.103 g) than WWM-21 (0.102 g) and WWM-66 (0.094 g) at [-0.19 MPa; 38 °C] (Figure 6.3l).

At 26 °C, increase root dry mass (RDM) among landraces was linked with lowering osmotic potential of the water agar (Figure 6.3m-n). At [-0.05 MPa; 26 °C], highest RDM (0.104 g) was recorded in WWM-09 and the lowest RDM (0.094 g) was recorded in WWM-21 (Figure 6.3m). Accessions WWM-09 and WWM-21 recorded higher RDM values (0.140 and 0.130 g) than WWM-66 and WWM-46 (0.129 and 0.114 g) at [-0.09 MPa; 26 °C] (Figure 6.3n). At [-0.19 MPa; 26 °C], highest RDM (0.155 g) was recorded in WWM-09 and WWM-21, while the lowest RDM (0.139 g) was recorded in WWM-46 (Figure 6.3o). At 38 °C, RDM decreased with increasing negativity of the osmotic potential across landrace accessions evaluated (Figure 6.3p-r). At [-0.05 MPa; 38 °C], RDM ranged from 0.076 – 0.082 g (Figure 6.3p). Figure 6.3q shows the RDM range of 0.051 - 0.059 g at [-0.09 MPa; 38 °C]. At higher temperature (38 °C) and lowest osmotic potential (-0.19 MPa), RDM ranged from 0.031 to 0.033 g (Figure 6.3r).

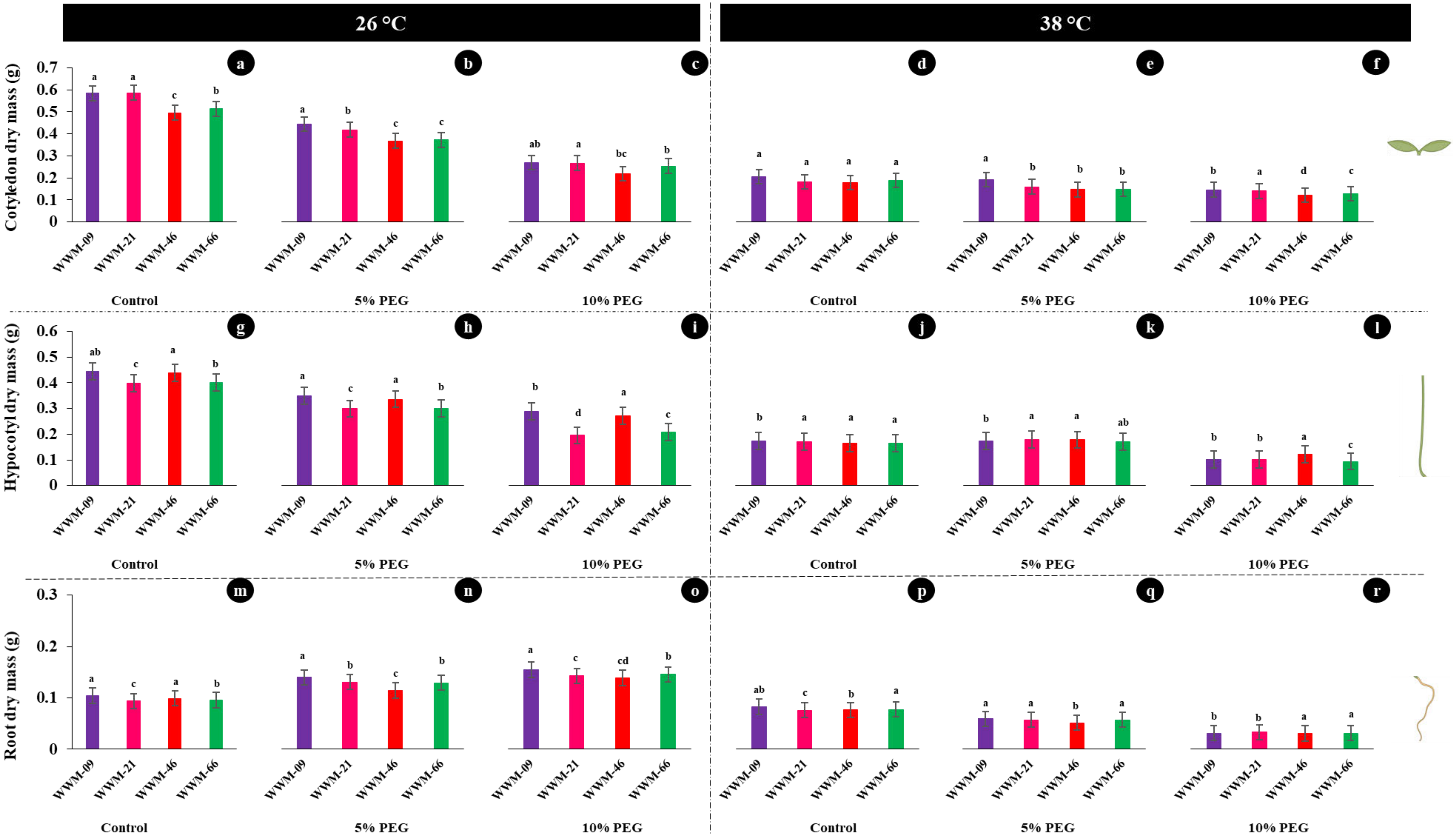


Figure 6.3: Dry mass of citron watermelon seedling axis (cotyledon, hypocotyl and roots) at day five after exposure to osmotic stress and heat stress

6.3.3 *Relative water content (RWC)*

The analysis of variance showed significant differences in RWC for single factors (genotype, osmotic potential and temperature) ($P < 0.001$). Significant treatment interactions were observed for treatment combinations; genotype × temperature and osmotic potential × temperature ($P < 0.05$) (Table 6.3). At [-0.05 MPa; 26 °C], highest RWC (90.03%) was recorded in WWM-09 and the lowest RWC (81.19%) was recorded in WWM-46. Highest and lowest RWC were recorded in WWM-09 (82.98%) and WWM-66 (70.89%) respectively at [-0.09 MPa; 26 °C]. Accessions WWM-09 (72.72%) and WWM-21 (65.18%) had highest and lowest RWC at [-0.19 MPa; 26 °C], respectively. A continuous decrease in RWC of the embryonic leaf was observed with increasing temperature and osmotic potential negativity. At [-0.05 MPa; 38 °C], RWC ranged from 73.04% in WWM-66 to 84.05% in WWM-09. Accessions WWM-09 (75.36%) and WWM-66 (65.00%) had highest and lowest RWC at [-0.09 MPa; 38 °C], respectively. At [-0.19 MPa; 38 °C], RWC was highest in WWM-09 (67.21%) and lowest in WWM-66 (52.93%) (Table 6.4).

6.3.4 *Photosynthetic pigments*

There were significant differences and significant treatment interactions in *Chl a*, *Chl b*, *Chl a+b*, *Chl a/b* and carotenoids among citron watermelon genotypes ($P < 0.05$) (Table 6.3). At [-0.05 MPa; 26 °C], *Chl a* ranged from 3.299 to 4.159 mg g^{-1}, *Chl b* ranged from 1.238 to 1.298 mg g^{-1}, *Chl a+b* ranged from 4.565 to 5.444 mg g^{-1}; *Chl a/b* ranged from 2.632 to 3.237, and carotenoids ranged from 0.938 to 0.955 mg g^{-1}. Highest values of *Chl a* (3.508 mg g^{-1}), *Chl b* (1.120 mg g^{-1}), *Chl a+b* (4.628 mg g^{-1}), *Chl a/b* (3.158) and carotenoids (0.908 mg g^{-1}) were recorded in WWM-09—while lowest values of *Chl a* (2.800 mg g^{-1}), *Chl b* (0.912 mg g^{-1}), *Chl a+b* (3.738 mg g^{-1}), *Chl a/b* (2.775) and carotenoids (0.851 mg g^{-1}) were recorded in WWM-46, WWM-66, WWM-66, WWM-46 and WWM-46 at [-0.09 MPa; 26 °C] respectively. At [-0.19 MPa; 26 °C], highest *Chl a* (2.875 mg g^{-1}), *Chl b* (0.839 mg g^{-1}), *Chl a+b* (3.702 mg g^{-1}), *Chl a/b* (3.480) and carotenoids (0.637 mg g^{-1}) were recorded in WWM-09, WWM-66, WWM-09, WWM-09 and WWM-09 respectively—lowest *Chl a* (2.114 mg g^{-1}), *Chl b* (0.769 mg g^{-1}), *Chl a+b* (2.897 mg g^{-1}), *Chl a/b* (2.861) and carotenoids (0.499 mg g^{-1}) were recorded in WW-46, WW-21, WW-46, WW-66 and WW-66 (Table 6.5).

At [-0.05 MPa; 38 °C], highest *Chl a* (2.427 mg g^{-1}), *Chl b* (0.840 mg g^{-1}), *Chl a+b* (3.267 mg g^{-1}), *Chl a/b* (3.325) and carotenoids (0.755 mg g^{-1}) were recorded in WWM-21, WWM-21, WWM-21, WWM-09 and WWM-46 respectively—lowest *Chl a* (2.200 mg g^{-1}), *Chl b* (0.717 mg g^{-1}), *Chl a+b* (2.921 mg g^{-1}), *Chl a/b* (3.079) and carotenoids (0.718 mg g^{-1}) were recorded in WW-66, WW-46, WW-46, WW-46 and WW-66. At [-0.09 MPa; 38 °C], highest *Chl a* (1.869 mg g^{-1}), *Chl b* (0.614 mg g^{-1}), *Chl a+b* (2.483 mg g^{-1}), *Chl a/b* (3.078) and carotenoids (0.594 mg g^{-1}) were recorded in WWM-21, WWM-21, WWM-21, WWM-09 and WWM-09 respectively—lowest *Chl a* (1.773 mg g^{-1}), *Chl b* (0.541 mg g^{-1}), *Chl a+b* (2.133 mg g^{-1}), *Chl a/b* (2.944) and carotenoids (0.460 mg g^{-1}) were recorded in WW-09, WW-46, WW-46, WW-46 and WW-66. At [-0.19 MPa; 38 °C], highest *Chl a* (1.344 mg g^{-1}), *Chl b* (0.452 mg g^{-1}), *Chl a+b* (1.797 mg g^{-1}), *Chl a/b* (3.037) and carotenoids (0.529 mg g^{-1}) were recorded in WWM-21, WWM-21, WWM-21, WWM-66 and WWM-09 respectively—lowest *Chl a* (1.022 mg g^{-1}), *Chl b* (0.351 mg g^{-1}), *Chl a+b* (1.373 mg g^{-1}), *Chl a/b* (3.000) and carotenoids (0.316 mg g^{-1}) were recorded in WW-46, WW-46, WW-46, WW-09 and WW-66 (Table 6.5).

Table 6.3: Analysis of variance with mean squares and significant tests of relative water content and photosynthetic pigments of four citron watermelon genotypes under varying temperature and osmotic potential after five days of treatment exposure

Source of variation	**d.f**	**RWC**	***Chl (a)***	***Chl (b)***	***Chl (a+b)***	***Chl (a/b)***	**Carotenoids**
Genotype (G)	3	316.979**	0.854**	0.009	1.014**	0.532**	0.029**
Osmotic potential (OP)	2	1662.182**	8.241**	1.084**	15.299**	0.024**	0.808**
Temperature (T)	1	1152.240**	29.022**	3.680**	53.371**	0.079	1.199**
G ×OP	6	9.820	0.006	0.007	0.005	0.082	0.008**
G × T	3	48.277*	0.401**	0.014	0.523**	0.142*	0.006**
OP × T	2	25.636*	0.007	0.011	0.034	0.040	0.072**
G × O × T	6	16.933	0.003*	0.006*	0.010*	0.087*	0.003**
Residual	48	8.176	0.019	0.006	0.035	0.038	0.017

$*P < 0.05$, $**P < 0.001$, **d f**; degrees of freedom, **RWC**; relative water content, ***Chl (a)***; chlorophyll *a*, ***Chl (b)***; chlorophyll *b*, ***Chl (a+b)***; total chlorophyll, ***Chl (a/b)***; ratio of *Chl (a)* to *Chl (b)*

Table 6.4: Means for percentage relative water content of citron watermelon embryonic leaf under varying temperatures and osmotic potential

	Relative water content (%)					
	Temperature (26 °C)			Temperature (38 °C)		
Genotype	**Control**	**5% PEG**	**10% PEG**	**Control**	**5% PEG**	**10% PEG**
WWM-09	**90.03^{a}**	**82.98^{a}**	**73.72^{a}**	**84.05^{a}**	**75.36^{a}**	**67.21^{a}**
WWM-21	84.14^{b}	74.12^{b}	**65.18^{b}**	79.04^{b}	70.74^{b}	61.13^{b}
WWM-46	**81.19^{c}**	76.70^{b}	67.99^{b}	74.12^{c}	67.61^{c}	55.99^{c}
WWM-66	83.49^{bc}	**70.89^{c}**	71.80^{a}	**73.04^{c}**	**65.00^{c}**	**52.93^{d}**
cv%	3.900	4.800	6.300	4.100	7.400	5.300
P-value	<0.001	0.031	0.024	0.017	0.011	<0.001

Means in the same column followed by the same letter are not significantly different, while figures with different superscript letter are significantly different according to Fisher's test

Table 6.5: Mean values for photosynthetic pigments (chlorophyll and carotenoids) under varying temperatures and osmotic potential

		Chlorophyll (*a*) (mg g^{-1} DW)			**Chlorophyll (*b*) (mg g^{-1} DW)**			**Chlorophyll (*a*+*b*) (mg g^{-1} DW)**			**Chlorophyll (a/b)**			**Carotenoids (mg g^{-1})**		
Temp (°C)	**Genotype**	Control	5% PEG	10% PEG	Control	5% PEG	10% PEG	Control	5% PEG	10% PEG	Control	5% PEG	10% PEG	Control	5% PEG	10% PEG
26	WWM-09	**4.159^{a}**	**3.508^{a}**	**2.875^{a}**	1.285^{a}	**1.120^{a}**	0.827^{a}	**5.444^{a}**	**4.628^{a}**	**3.702^{a}**	**3.237^{a}**	**3.158^{a}**	**3.480^{a}**	**0.946^{a}**	**0.908^{a}**	**0.637^{a}**
	WWM-21	3.580^{b}	2.912^{ab}	2.363^{b}	1.238^{c}	1.040^{a}	**0.769^{b}**	4.819^{b}	3.951^{b}	3.132^{b}	2.897^{b}	2.802^{b}	3.073^{b}	**0.938^{a}**	0.893^{ab}	0.543^{b}
	WWM-46	**3.299^{c}**	**2.800^{b}**	**2.114^{b}**	**1.266^{b}**	**1.001^{ab}**	0.782^{b}	**4.565^{cd}**	3.810^{c}	2.897^{c}	2.635^{c}	**2.775^{bc}**	2.707^{c}	**0.955^{a}**	**0.851^{b}**	0.600^{ab}
	WWM-66	3.374^{c}	2.827^{b}	2.246^{b}	**1.298^{a}**	0.912^{b}	**0.839^{a}**	4.673^{c}	**3.738^{d}**	**3.084^{b}**	**2.632^{c}**	3.108^{ab}	**2.681^{d}**	**0.914^{a}**	0.895^{a}	**0.499^{c}**
	cv%	32.100	19.400	11.900	22.800	30.200	20.000	21.100	8.200	11.700	25.800	17.700	22.300	7.100	13.500	15.400
	P-value	0.027	0.016	0.041	0.028	0.045	0.017	0.011	0.018	0.031	0.040	0.035	0.019	0.087	0.022	0.037
38	WWM-09	2.407^{a}	**1.773^{b}**	1.222^{b}	0.726^{b}	0.576^{b}	0.407^{ab}	3.132^{b}	2.349^{b}	1.629^{b}	**3.325^{a}**	**3.078^{a}**	**3.000^{a}**	0.749^{a}	**0.594^{a}**	**0.529^{a}**
	WWM-21	**2.427^{a}**	**1.869^{a}**	**1.344^{a}**	**0.840^{a}**	**0.614^{a}**	**0.452^{a}**	**3.267^{a}**	**2.483^{a}**	**1.797^{a}**	2.912^{c}	3.045^{a}	2.974^{ab}	0.735^{a}	0.478^{b}	0.346^{b}
	WWM-46	2.204^{b}	1.592^{d}	**1.022^{d}**	**0.717^{b}**	**0.541^{bc}**	**0.351^{b}**	**2.921^{d}**	**2.133^{d}**	**1.373^{d}**	**3.079^{b}**	**2.944^{b}**	2.918^{b}	**0.755^{a}**	0.476^{b}	0.325^{b}
	WWM-66	**2.200^{b}**	1.671^{c}	1.088^{c}	0.814^{a}	0.565^{b}	0.358^{b}	3.014^{c}	2.236^{c}	1.447^{c}	2.711^{d}	2.960^{b}	**3.037^{a}**	**0.718^{b}**	**0.460^{b}**	**0.316^{b}**
	cv%	24.600	13.400	17.400	27.000	16.800	29.600	17.230	13.600	9.000	12.300	18.720	9.200	11.600	32.000	16.300
	P-value	0.044	<.001	0.036	0.048	0.038	0.040	<.001	<.001	<.001	0.013	0.048	0.028	0.050	0.035	0.048

Means in the same column followed by the same letter are not significantly different, while figures with different superscript letter are significantly different according to Fisher's test

Table 6.6: Analysis of variance with mean squares and significant tests of non-structural carbohydrates of four citron watermelon genotypes under varying temperature and osmotic potential after five days of treatment exposure

S.O.V	**d.f**	**TSS**$_{\text{Cotyledon}}$	**TSS**$_{\text{Hypocotyl}}$	**TSS**$_{\text{Roots}}$	**Starch**$_{\text{Cotyledon}}$	**Starch**$_{\text{Hypocotyl}}$	**Starch**$_{\text{Roots}}$	**NRS**$_{\text{Cotyledon}}$	**NRS**$_{\text{Hypocotyl}}$	**NRS**$_{\text{Roots}}$	**MDA**$_{\text{Cotyledon}}$	**MDA**$_{\text{Hypocotyl}}$	**MDA**$_{\text{Roots}}$
Genotype (G)	3	1394.566**	12424.07**	4548.730**	$2.617\times10^{-3**}$	$3.172\times10^{-3**}$	$5.071\times10^{-5*}$	2.071**	48.684**	490.234**	5.253**	1.194**	10.685**
Osmotic potential (OP)	2	$5.592\times10^{4**}$	118489.79**	$1.047\times10^{6**}$	$3.792\times10^{-3**}$	1.282×10^{-4}	$1.366\times10^{-3**}$	76.060**	261.225**	21380.618**	27.553**	8.372**	77.109**
Temperature (T)	1	6387.359**	20749.19**	49957.650**	0.530**	$9.844\times10^{-3**}$	$5.163\times10^{-4**}$	40.690**	2180.426**	10713.530**	16.044**	11.595**	12.405**
G × OP	6	1174.187**	7359.55**	22848.410**	1.837×10^{-4}	1.690×10^{-5}	1.102×10^{-5}	2.357**	14.794**	322.430**	0.343**	0.109**	0.284**
G × T	3	14.858	31.72	71.640	$1.184\times10^{-3**}$	5.175×10^{-5}	3.374×10^{-7}	0.061	19.759**	35.503**	0.624**	0.087*	0.401**
OP × T	2	245.297**	120.47	1050.970**	0.140**	$7.042\times10^{-4**}$	2.136×10^{-6}	1.302**	304.044**	625.448**	0.130*	0.631**	0.158*
G × OP ×T	6	24.729*	57.62	193.340*	$4.117\times10^{-4**}$	2.345×10^{-5}	1.735×10^{-6}	0.406**	6.206**	15.130*	0.179**	0.002	0.053
Residual	48	7.299	57.36	79.990	9.115×10^{-5}	5.349×10^{-5}	9.105×10^{-6}	0.042	0.717	4.892	0.024	0.016	0.033

* $P < 0.05$, **$P < 0.001$, **S.O.V**; Source of Variation, **d f**; Degrees of freedom, **TSS**; Total Soluble Sugars, **NRS**; Non-Reducing Sugars, **MDA**; Malondialdehyde

6.3.5 Non-structural carbohydrates

The TSS quantified in cotyledon, hypocotyl and roots increased with increasing PEG concentration at 26 °C and 38 °C (Figure 6.4a-f). The highest TSS was recorded in WWM-46 (718.000 µmol g^{-1} DW) at [-0.05 MPa; 26 °C] in the root (Figure 6.4c), while the lowest TSS (135.490 µmol g^{-1} DW) were recorded in cotyledons of WWM-09 at [-0.05; 38 °C] (Figure 6.4d).

Starch content accumulated in the embryonic leaf, hypocotyl and roots of evaluated citron watermelon accessions significantly differ ($P < 0.05$) under varying osmotic potentials and temperature (Table 6.6). Highest (≥ 0.589 mg $part^{-1}$) starch content was accumulated in the embryonic leaf in WWM-46 and WWM-66 at [-0.19 MPa; 26 °C] (Figure 6.4g). The starch in cotyledons, hypocotyl and roots all decreased with increasing PEG concentration and temperature, with the greatest reductions occurring under the highest water stress (-0.19 MPa) (Figure 6.4g-l).

The non-reducing sugars (NRS) accumulated in the cotyledon, hypocotyl and roots of evaluated citron watermelon accession significantly differ ($P < 0.05$) under varying osmotic potentials and temperature. Highest NRS (≥ 84.093 µmol g^{-1} DW) was recorded under [-0.05 MPa; 26 °C] in the roots, while lowest NRS (4.387 µmol g-1 DW) was recorded at [-0.19 MPa; 38 °C] in the cotyledons.

6.3.6 Malondialdehyde (MDA)

The ANOVA revealed genotype, temperature, osmotic potential, seedling axis and their interactions were statistically significant for MDA. Highest MDA levels (11.65 µmol g^{-1} FW) were recorded in roots at [-0.19 MPa; 38 °C] in WWM-66. The lowest MDA (≤ 2.04 µmol g-1 FW) was recorded in WWM-09 and WWM21 in the hypocotyl at [-0.05 MPa; 26°C] (Figure 6.4).

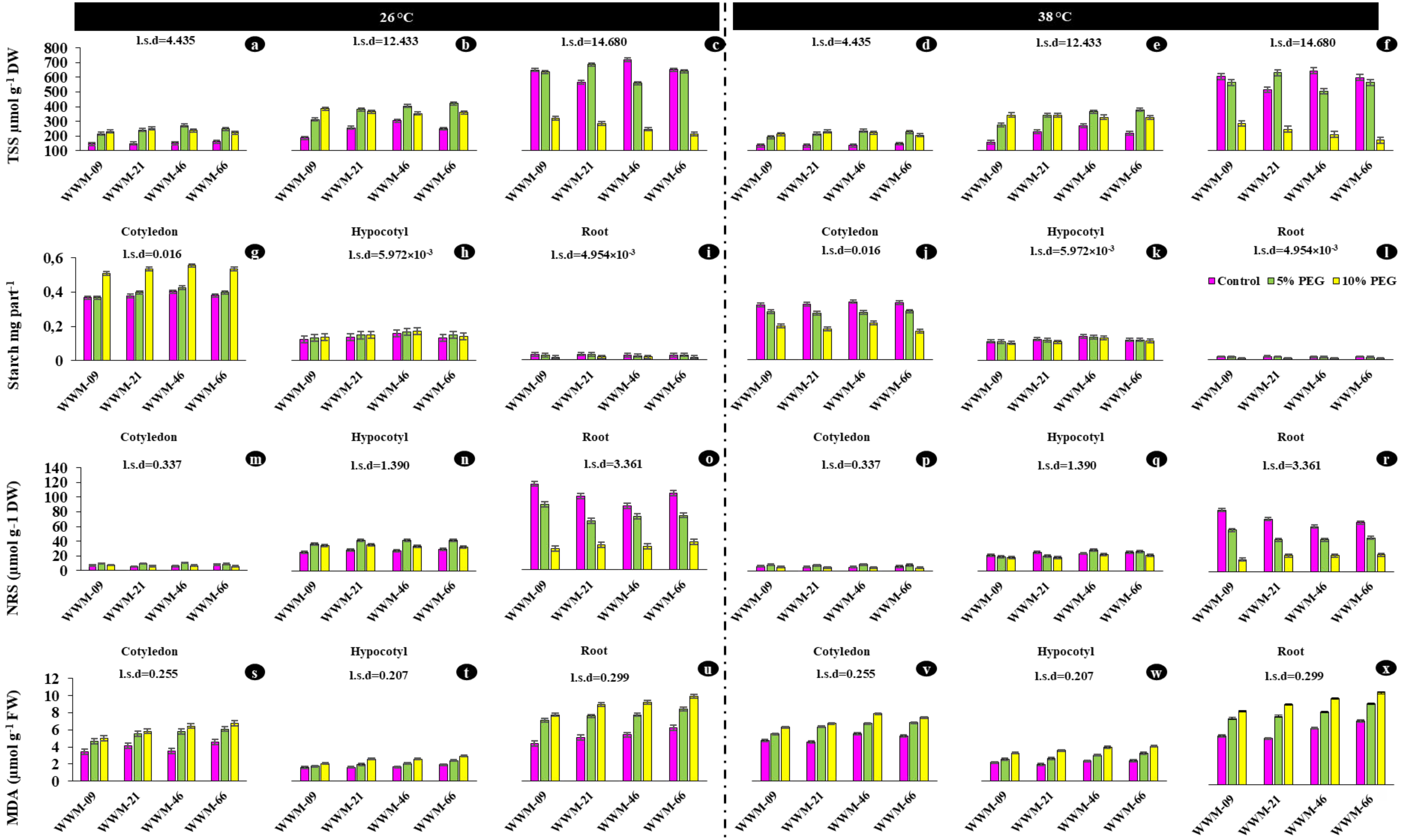


Figure 6.4: Total soluble solutes (**a-f**), starch (**g-l**), non-reducing sugars (**m-r**) and malondialdehyde (**s-x**)of citron watermelon seedling axis (cotyledon, hypocotyl and roots) at day five after exposure to osmotic and heat stress

6.3.7 Phytosterols

ANOVA revealed genotype, temperature, osmotic potential, seedling axis and their interactions were statistically significant for stigmasterol, sitosterol, campesterol and total phytosterol Table 6.7.

Table 6.7: Analysis of variance showing mean squares and significant tests for phytosterols (stigmasterol, sitosterol and campesterol) of 4 citron watermelon landrace accessions evaluated under combined stress (heat and osmotic stress)

Source of variation	**d.f.**	**Stigmasterol**	**Sitosterol**	**Campesterol**	**Total Phytosterol**
Genotype (G)	3	0.014^{ns}	0.002^{ns}	0.016^{*}	0.082^{ns}
Temperature (T)	1	7.835^{**}	7.882^{**}	5.581^{**}	63.507^{**}
Osmotic potential (OP)	2	0.634^{**}	0.389^{**}	0.788^{**}	5.249^{**}
Seedling axis (SA)	1	4.929^{**}	1.679^{**}	3.453^{**}	28.882^{**}
G × T	3	0.003^{*}	0.004^{**}	0.003^{ns}	0.013^{ns}
G × OP	6	0.003^{ns}	0.003^{ns}	0.003^{ns}	0.006^{ns}
T × OP	2	0.237^{**}	0.172^{**}	0.039^{*}	1.185^{**}
G × SA	3	0.002^{ns}	0.001^{ns}	0.004^{*}	0.007^{ns}
T × SA	1	0.767^{**}	0.935^{**}	0.488^{**}	6.460^{**}
OP × SA	2	0.163^{**}	0.188^{**}	0.024^{*}	0.752
G × T × OP	6	0.005^{ns}	0.003^{ns}	0.006^{*}	0.022^{ns}
G × T × SA	3	0.005^{ns}	0.004	0.003^{*}	0.020^{ns}
G × OP × SA	6	0.004^{ns}	0.009^{ns}	0.003^{*}	0.030^{ns}
T × OP × SA	2	0.296^{**}	0.130^{**}	0.148^{**}	1.471^{**}
G × T × OP × SA	6	0.003^{*}	0.004^{*}	0.008^{*}	0.020^{ns}
Residual	96	0.013	0.010	0.017	0.067

* and ** denote significant at 5 and 1% probability levels, respectively. ns, non-significant

At 26 °C, highest stigmasterol was recorded in the roots of WWM-09 (0.535 mg g^{-1} DW) at -0.19 MPa. The lowest stigmasterol (≤ 0.095 mg g^{-1} DW) was recorded in the embryonic leaves of WWM-21 and WWM-46 at -0.05 MPa. Highest sitosterol (≥ 0.258 mg g^{-1} DW) was recorded in roots of WWM-09 and WWM-21 at -0.19 MPa. Lowest sitosterol concentration was recorded in roots of WWM-09 (0.109 mg g^{-1} DW) at -0.05 MPa. Highest campesterol (≥ 0.563 mg g^{-1} DW) was recorded in roots of WWM-09 and WWM-46 at -0.19 MPa (Table 6.8).

At 38 °C, highest stigmasterol was recorded in the roots of WWM-09 (1.003 mg g^{-1} DW) at -0.19 MPa. The lowest stigmasterol (0.225 mg g^{-1} DW) was recorded in the cotyledon of WWM-21 at -0.05 MPa. Highest sitosterol (0.886 mg g^{-1} DW) was recorded in roots of WWM-09 at -0.19 MPa. Lowest sitosterol concentration was recorded in cotyledon of WWM-21 and WWM-46 (≤ 0.339 mg g^{-1} DW) at -0.05 MPa. Highest campesterol (≥ 0.899 mg g^{-1} DW) was recorded in roots of WWM-21 and WWM-46 at -0.19 MPa, while the lowest campesterol (0.216 mg g^{-1} DW) was recorded in the cotyledon of WWM-66 at -0.05 MPa (Table 6.8).

Table 6.8: Mean values for stigmasterol, sitosterol and campesterol in citron watermelon seedling axis (cotyledon and roots) under different temperature and osmotic potential

		Cotyledon									**Root**								
		[-0.05 MPa]			**[-0.09 MPa]**			**[-0.19 MPa]**			**[-0.05 MPa]**			**[-0.09 MPa]**			**[-0.19 MPa]**		
Temperature	**Genotype**	***Stig***	***Sito***	***Camp***	***Stig***	***Sito***	***Camp***	***Stig***	***Sito***	***Camp***	***Stig***	***Sito***	***Camp***	***Stig***	***Sito***	***Camp***	***Stig***	***Sito***	***Camp***
26 °C	WWM-09	0.103^{a}	0.123^{b}	0.075^{a}	0.180^{a}	0.143^{a}	0.201^{b}	0.205^{c}	0.160^{c}	0.241^{b}	0.340^{b}	0.109^{c}	0.281^{a}	0.375^{a}	0.223^{a}	0.305^{c}	0.535^{a}	0.258^{a}	0.617^{a}
	WWM-21	0.080^{bc}	0.115^{c}	0.063^{b}	0.160^{bc}	0.113^{c}	0.196^{c}	0.216^{b}	0.183^{a}	0.242^{b}	0.309^{c}	0.137^{b}	0.242^{b}	0.281^{d}	0.207^{b}	0.301^{c}	0.434^{c}	0.262^{a}	0.488^{c}
	WWM-46	0.095^{b}	0.123^{b}	0.078^{a}	0.153^{c}	0.125^{b}	0.195^{c}	0.193^{d}	0.163^{c}	0.238^{c}	0.313^{c}	0.145^{b}	0.211^{c}	0.348^{bc}	0.227^{a}	0.355^{b}	0.461^{b}	0.238^{b}	0.414^{d}
	WWM-66	0.103^{a}	0.135^{a}	0.068^{b}	0.173^{ab}	0.138^{ab}	0.216^{a}	0.236^{a}	0.173^{b}	0.293^{a}	0.410^{a}	0.152^{a}	0.250^{b}	0.355^{b}	0.148^{c}	0.395^{a}	0.422^{d}	0.246^{b}	0.563^{b}
	cv%	22.600	12.400	19.300	14.200	11.900	19.40	17.400	11.400	34.300	32.900	22.800	20.000	18.400	22.400				
	P-value	0.032	<.001	0.017	<.001	0.041	0.038	0.038	0.015	0.027	<.001	0.035	0.047	0.044	0.027	0.039	0.021	0.021	<.001
38 °C	WWM-09	0.314^{a}	0.424^{a}	0.301^{a}	0.479^{b}	0.441^{a}	0.488^{a}	0.713^{bc}	0.552^{c}	0.658^{b}	0.823^{a}	0.505^{d}	0.720^{c}	1.240^{b}	1.059^{b}	1.059^{a}	1.003^{a}	0.886^{a}	0.897^{b}
	WWM-21	0.225^{c}	0.331^{c}	0.250^{b}	0.429^{c}	0.407^{b}	0.428^{c}	0.725^{b}	0.577^{a}	0.666^{b}	0.733^{c}	0.567^{b}	0.768^{b}	1.335^{a}	1.135^{a}	0.969^{b}	0.963^{c}	0.775^{c}	0.927^{a}
	WWM-46	0.301^{b}	0.369^{b}	0.242^{b}	0.390^{a}	0.390^{c}	0.454^{b}	0.700^{c}	0.539^{d}	0.598^{c}	0.740^{c}	0.547^{c}	0.720^{c}	1.142^{d}	0.976^{c}	0.899^{b}	0.969^{c}	0.865^{b}	0.899^{b}
	WWM-66	0.301^{b}	0.339^{c}	0.216^{c}	0.424^{c}	0.446^{a}	0.454^{b}	0.743^{a}	0.560^{b}	0.675^{a}	0.789^{b}	0.657^{a}	0.775^{a}	1.218^{c}	1.052^{b}	1.045^{a}	0.976^{b}	0.879^{b}	0.865^{c}
	cv%	19.900	15.400	8.200	18.600	12.800	23.100	9.900	19.200	18.200	32.400	22.600	21.100	17.300	32.100				
	P-value	0.009	0.019	0.005	0.008	<.001	0.032	0.027	<.001	0.039	0.015	<.001	<.001	0.031	<.001	0.036	0.017	0.020	<.001

Stig; Stigmasterol (mg g^{-1} DW), **Sito**; Sitosterol (mg g^{-1} DW), **Camp**; Campesterol (mg g^{-1} DW)

6.3.8 Principal component analysis (PCA) for assessed traits

Table 6.9 shows the PCA with factor loadings, eigenvalues, and percent variance for the evaluated traits. Under [-0.05 MPa; 26 °C], PC1 accounted for 55.89% of the total variation and was positively correlated with CDM, RDM, RWC, *Chl a*, NRS and campesterol. Principal component 2 was positively correlated with ADGR, HDM, starch and carotenoids, contributing to 27.72% of the total variation. PC3 accounted for 16.39% of the total variation and was positively correlated with HDM, RDM and stigmasterol.

At [-0.09 MPa; 26 °C], PC1 accounted for 59.30% of the total variation and was positively correlated with RWC, *Chl a*, *Chl b*, *Chl (a + b)* and sitosterol. PC 2 was positively correlated with ADGR, RDM and carotenoids, contributing to 24.24% of the total variation. Stigmasterol and campesterol were positively correlated to PC3 which accounted for 16.47% of the total variation (Table 6.9).

Under [-0.19 MPa; 26 °C], RDM, *Chl a*, *Chl (a + b)*, *Chl a/b* and stigmasterol were positively correlated to PC1 which accounted for 55.65% of the total variation. PC 2 was positively correlated with CDM and sitosterol, contributing to 26.08% of the total variation. Relative water content and *Chl b* were positively correlated to PC3 accounting for 18.27% of the total variation (Table 6.9).

At [-0.05 MPa; 38 °C], PC1 accounted for 47.96% of the total variation and was positively correlated with RWC, *Chl a*, *Chl (a + b)*, NRS and campesterol. PC 2 was positively correlated with *Chl b*, contributing to 30.89% of the total variation. Stigmasterol and sitosterol were positively correlated to PC3 which accounted for 21.15% of the total variation (Table 6.9).

At [-0.09 MPa; 38 °C], PC1 accounted for 61.34% of the total variation and was positively correlated with CDM, HDM, RWC, Chl (a/b), stigmasterol and sitosterol. PC 2 was positively correlated with *Chl b* and TSS, contributing to 28.25% of the total variation. Campesterol and RDM were positively correlated to PC3 which accounted for 10.41% of the total variation (Table 6.9).

At [-0.19 MPa; 38 °C], PC1 accounted for 54.68% of the total variation and was positively correlated with CDM, *Chl a*, *Chl (a+b)*, TSS and campesterol. PC 2 was positively correlated with HDM and ADGR, contributing to 27.81% of the total variation. Carotenoids and starch were positively correlated to PC3 which accounted for 17.52% of the total variation (Table 6.9).

Table 6.9: Summary of factor loadings, eigenvalue, percent and cumulative variation for dry matter, pigments, non-structural carbohydrates, malondialdehyde and phytosterols among 4 citron watermelon accessions under varying temperature and osmotic potential

	[-0.05 MPa; 26 °C]			**[-0.09 MPa; 26 °C]**			**[-0.19 MPa; 26 °C]**			**[-0.05 MPa; 38 °C]**			**[-0.09 MPa; 38 °C]**			**[-0.19 MPa; 38 °C]**		
Traits	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3	PC 1	PC 2	PC 3
ADGR	0.658	0.682	0.318	0.273	0.920	-0.282	-0.280	0.895	-0.347	-0.178	-0.976	0.128	0.582	-0.685	-0.438	-0.654	0.749	-0.108
CDM	0.773	-0123	-0.623	0.870	0.269	-0.414	0.603	0.795	-0.067	0.715	-0.457	0.529	0.885	-0.460	0.070	0.984	-0.149	0.096
HDM	0.506	0.631	0.588	0.717	-0.633	0.292	0.584	-0811	-0.033	-0.536	-0.571	-0.622	0.969	-0.177	-0.171	0.135	0.985	0.105
RDM	0.746	0.286	0.602	0.718	0.694	-0.050	0.952	0.203	0.229	0.675	-0.648	0.352	0.825	0.167	0.539	0.704	0.453	-0.547
RWC	0.973	-0.225	0.053	0.917	-0.399	-0.015	0.694	-0.180	0.697	0.917	-0.348	-0.197	0.892	-0.368	-0.263	0.845	0.012	0.535
Chl a	0.999	-0.052	-0.008	0.992	0.108	0.061	0.983	0.156	-0.096	0.909	0.088	-0.408	0.839	0.526	-0.141	0.925	0.282	-0.256
Chl b	0.107	-0.514	0.851	0.882	-0.222	-0.415	0.469	0.066	0.881	0.157	0.986	0.063	0.700	0.700	-0.141	0.896	0.398	-0.197
Chl (a+b)	0.995	-0.087	0.048	0.999	0.030	-0.036	0.988	0.154	-0.008	0.817	0.483	-0.314	0.814	0.563	-0.141	0.918	0.311	-0.245
Chl (a/b)	0.987	0.033	-0.154	0.532	0.553	0.641	0.908	0.168	-0.384	0.450	-0.839	-0.307	0.996	-0.010	-0.083	0.325	-0.792	-0.517
Carotenoids	0.283	0.959	0.012	0.560	0.829	-0019	0.648	-0.637	-0.418	0.001	-0.789	-0.614	0.767	-0.639	0.058	0.672	-0.384	0.633
TSS	-0.671	0.455	0.585	-0.840	0.491	-0.232	0.750	0.122	-0.650	-0.926	-0.375	0.033	-0.377	0.926	0.022	0.810	0.323	0.489
Starch	-0.722	0.686	0.090	-0.836	-0.544	-0072	-0.853	-0.466	-0.234	-0.913	0.018	-0.407	-0.979	-0.157	-0.129	-0.548	0.427	0.719
NRS	0.728	-0.684	0.051	0.795	-0.144	0.589	-0.661	0.693	0.287	0.962	-0.153	0.227	-0.035	-0.893	0.449	-0.871	0.202	-0.448
MDA	-0.748	-0.663	0.031	-0.917	0.203	0.345	-0.885	0.097	0.456	-0.809	-0.108	0.578	-0.868	0.305	0.391	-0.912	0.007	-0.409
Stigmasterol	-0.067	-0.795	0.602	0.520	0.001	0.854	0.986	-0.156	0.064	0.172	-0.655	0.735	0.876	0.476	0.079	0.471	-0.826	-0.309
Sitosterol	-0.897	-0.360	0.257	0.713	-0.688	0.134	0.024	0.911	-0.411	-0.248	0.153	0.957	0.716	0.607	0.344	-0.259	-0.915	0.310
Campesterol	0.889	-0.395	0.233	-0.605	0.188	0.774	0.632	0.455	0.628	0.971	0.238	0.029	0.562	-0.259	0.786	0.881	0.107	-0.461
Eigenvalue	9.501	4.712	2.787	10.080	4.120	2.799	9.460	4.434	3.107	8.152	5.252	3.596	10.427	4.803	1.770	9.296	4.727	2.978
Variability (%)	55.891	27.718	16.392	59.296	24.237	16.467	55.646	26.080	18.274	47.955	30.893	21.152	61.337	28.253	10.410	54.680	27.805	17.515
Cumulative (%)	55.891	83.608	100	59.296	83.533	100	55.646	81.726	100	47.955	78.848	100	61.337	89.590	100	54.680	82.485	100

ADGR; average daily growth rate, **CDM**; cotyledon dry mass; **HDM**; hypocotyl dry mass; **RDM**; root dry mass; **RWC**; relative water content, ***Chl a***; Chlorophyll a, ***Chl b***; Chlorophyll b, ***Chl (a+b)***; total chlorophyll, **TSS**; total soluble solutes, **NRS**, non-reducing sugars, **MDA**; malondialdehyde

The PC biplots based on PCA analysis were used to picture the relationship among citron watermelon landraces based on evaluated parameters under varying temperature and osmotic stress (Figure 6.5a-f). Traits represented by parallel vectors or close to each other revealed a strong positive association, and those located nearly opposite (at 180°) showed highly negative association, while the vectors toward sides expressed a weak relationship.

At [-0.05 MPa; 26 °C], accessions WWM-21 and WWM-46 are grouped based on high starch and TSS. Accession WWM-09 is grouped based on high HDM and RDM. WWM-66 is grouped based on high sitosterol and MDA (Figure 6.5a). At [-0.09 MPa; 26 °C], WWM-66 and WWM-21 are grouped based on high campesterol, MDA and TSS (Figure 6.5b). In Figure 6.5c [-0.19 MPa; 26 °C], WWM-21 and WWM-66 are grouped based on high MDA, NRS and ADGR.

At [-0.05 MPa; 38 °C], WWM-66 was grouped based on high starch and sitosterol, WWM-21 is associated with high *Chl (a+b)*, campesterol and *Chla.* Accession WWM-46 and WWM-09 are associated with high (MDA, TSS and HDM) and (stigmasterol, *Chl(a/b)*, NRS and RWC) respectively (Figure 6.5d). At [-0.09 MPa; 38 °C], WWM-66 is associated with MDA and TSS, WWM-21 is associated with stigmasterol, RDM and *Chlb*. WWM-09 is associated with carotenoids, campesterol and RWC (Figure 6.5e). In Figure 6.5f, accession WWM-46 was associated with high starch, NRS and MDA. WWM-21 was associated with high RDM, campesterol, RWC and *Chla*. Accession WWM-09 was associated with high stigmasterol, CDM and *Chl(a/b).*

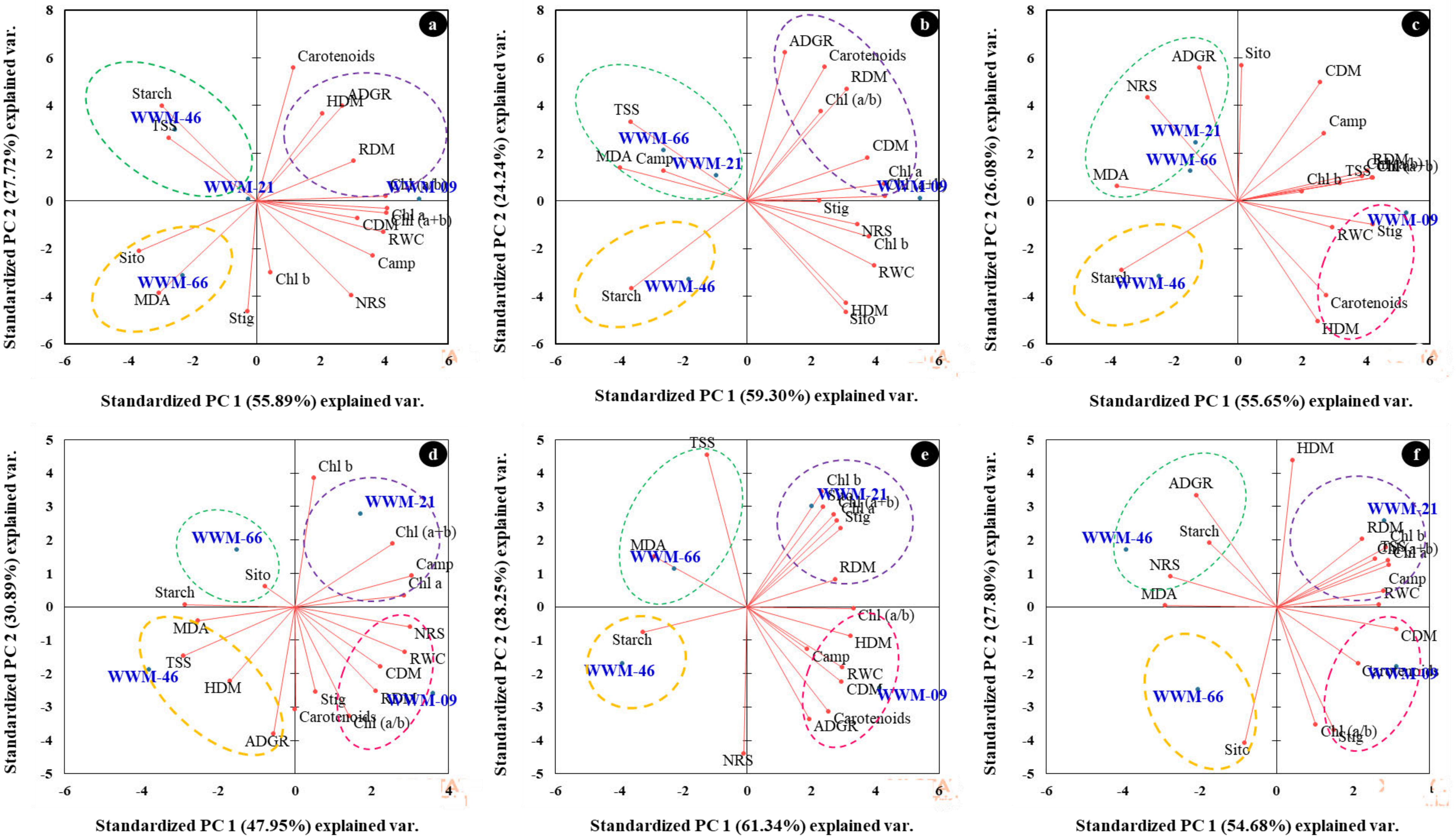


Figure 6.5: Principal component (PC) biplot of PC 1 vs PC 2 demonstrating the relationships among dry matter, pigments, non-structural carbohydrates, malondialdehyde and phytosterols of 4 citron watermelon accessions evaluated under (**a**) [-0.05 MPa; 26 °C], (**b**) [-0.09 MPa; 26 °C], (**c**) [-0.19 MPa; 26 °C], (**d**) [-0.05 MPa; 38 °C], (**e**) [-0.09 MPa; 38 °C] and (**f**) [-0.19 MPa; 38 °C]

6.4 Discussion

Water and heat stress act synergically under field conditions, making it challenging to define their contribution to drought stress in plants (Altunoglu et al., 2019; Francesca et al., 2021; Schwarz et al., 2010). Therefore, this experiment was conducted in a growth chamber at a controlled temperature and varied osmotic potential to identify the multiple abiotic stress-induced modifications in different phytosterols (campesterol, sitosterol and stigmasterol) in seedling axis (embryonic leaf and root) of genetically distinct citron watermelon accessions.

Combined stress affects biomass partitioning and growth more than the individual stresses of heat stress and drought stress (Meena et al., 2021). Our results showed that low osmotic potential (-0.19 MPa) and high temperature (38 °C) retarded seedling growth rate and dry matter accumulation in citron watermelon seedling axis (Figure 6.2; Table 6.2). The primary effect of drought stress is a decline in relative water content, and it is accompanied by changes in molecular, physiological, morphological and biochemical events and their complex interaction.

The genotypic response regarding dry matter allocation under all stress conditions varied significantly among citron watermelon accessions (Figure 6.3). Organ-specific translocation and allocation of dry matter is an important attribute for drought tolerance rather than total biomass production (Mandizvo et al., 2022b; Yang et al., 2012). Citron watermelon partitioned more carbon to roots than shoots under lowest osmotic potential (-0.19 MPa) (Figure 6.3), which could be attributed to the drought and heat stress tolerance ability of WWM-09 and WWM-46. Increased root biomass under drought will increase water and nutrient acquisition, an important mechanism of drought tolerance in citron watermelon (Mandizvo et al., 2022b).

Combined stress (drought and heat) severely impaired the photosynthetic system. Carotenoids, *Chla* and *Chlb* significantly declined under stress, particularly the combined stress (Table 6.5). These changes lead to the reactive oxygen species (ROS) generation, which causes the photoinhibition and oxidative injury of cellular components, such as the photosynthesis pigments (Hasanuzzaman et al., 2013; Mathur et al., 2014). Carotenoids are potential antioxidants during plant stress (McElroy and Kopsell, 2009). They act as light harvesters, quenchers and scavengers of triplate state chlorophylls and singlet oxygen species, dissipation of excess energy during stress and membrane stabilisers (Uarrota et al., 2018). Excess ROS production under drought and heat stress leads to oxidative damage in cells, with consequent inhibition of photosynthesis, damage of cellular structures, growth reduction and premature senescence (Hajihashemi and Sofo, 2018; Ul Hassan et al., 2021; Wahid et al., 2007).

Lipid peroxidation, an important criterion to evaluate the negative effects of stress on cell membrane, can be indirectly measured by malondialdehyde (MDA) content and electrolyte leakage. Combined stress reduced non-structural carbohydrates concentration in cotyledons and hypocotyl except for roots (Figure 6.4). Different stresses significantly reduced starch, TSS and NRS concentration, relative to the control.

Increased production of phytosterols imparted cross-tolerance to combined stress of heat and drought (Figure 6.6). Under different stress combinations, we observed relatively higher expression of campesterol in the cotyledon of WWM-09 and WWM-21 (Table 6.8). Campesterol is a precursor of oxidized steroids acting as growth hormones collectively named brassinosteroids (BRs). Ahammed et al. (2015) reported that brassinosteroids induce stress tolerance to abiotic stresses (high temperature, chilling, drought, salinity and heavy metals).

In Figure 6.6e [-0.09 MPa; 38 °C], stigmasterol was highly associated with *Chla* and *Chlb*. Stigmasterols are involved in transmembrane signal transduction into cells through formation of lipid microdomains in the membranes. Such microdomains "l*ipid rafts*", serve as anchoring platforms for signaling enzyme complexes (Valitova et al., 2016). In addition to membrane architectural function, sitosterol affect the activity of integral membrane proteins, including enzymes, ion channels, receptors and components of signal transduction pathways such as ATPases (Rogowska and Szakiel, 2020).

6.5 Conclusion

Drought stress reduced the relative water content and membrane stability, affecting overall plant growth. The tolerant accessions maintained significantly higher growth rate and biomass under combined stress (heat and drought) than the sensitive accessions, mainly due to protection of photosynthetic pathway. The combined stress also increased osmolyte concentration and antioxidative compounds in tolerant accessions. The results confirm that campesterol is a major component of the sterol fraction of citron watermelon embryonic leaves. Considering different genotypes and treatment conditions, variations in sterol composition are dependent on both the genotype and environmental factors, while changes in the main lipid classes are mainly determined by genetic background. During exposure to stress, citron watermelon tends to accumulate specific sterols, suggesting that sterols have a prominent role in plants' tolerance to stress. It was also found out that increased synthesis of stigmasterol during heat/ drought may be associated with inbred stress resistance of citron watermelon. The characteristics of phytosterol changes in examined accessions allowed the selection of interesting citron watermelon genotypes, i.e. WWM-09 and WWM-21 to be chosen for in-depth examination. In fact, additional research on other treatments such as salinity as well as other parameters (lipid peroxidation) could be investigated and monitored. The relation of campesterol level in WWM-09 and WWM-21 with respect stigmasterol content is another aspect that would be worthwhile examining.

References

Ahammed G.J., Xia X.J., Li X., Shi K., Yu J.Q., Zhou Y.H. (2015) Role of brassinosteroid in plant adaptation to abiotic stresses and its interplay with other hormones. Curr Protein Pept Sci 16:462-73. DOI: 10.2174/1389203716666150330141427.

Akashi K., Miyake C., Yokota A. (2001) Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. FEBS Letters 508:438-442. DOI: 10.1016/s0014-5793(01)03123-4.

Altunoglu Y.C., Keles M., Can T.H., Baloglu M.C. (2019) Identification of watermelon heat shock protein members and tissue-specific gene expression analysis under combined drought and heat stresses. Turk J Biol 43:404-419. DOI: 10.3906/biy-1907-5.

Ansari W.A., Atri N., Ahmad J., Qureshi M.I., Singh B., Kumar R., Rai V., Pandey S. (2019) Drought mediated physiological and molecular changes in muskmelon (*Cucumis melo* L.). PLoS One 14:e0222647. DOI: 10.1371/journal.pone.0222647.

Cicevan R., Al Hassan M., Sestras A.F., Prohens J., Vicente O., Sestras R.E., Boscaiu M. (2016) Screening for drought tolerance in cultivars of the ornamental genus Tagetes (Asteraceae). PeerJ 4:e2133. DOI: 10.7717/peerj.2133.

Correa S.M., Fernie A.R., Nikoloski Z., Brotman Y. (2020) Towards model-driven characterization and manipulation of plant lipid metabolism. Prog Lipid Res 80:101051. DOI: 10.1016/j.plipres.2020.101051.

Demirel U., Morris W.L., Ducreux L.J.M., Yavuz C., Asim A., Tindas I., Campbell R., Morris J.A., Verrall S.R., Hedley P.E., Gokce Z.N.O., Caliskan S., Aksoy E., Caliskan M.E., Taylor M.A., Hancock R.D. (2020) Physiological, Biochemical, and Transcriptional Responses to Single and Combined Abiotic Stress in Stress-Tolerant and Stress-Sensitive Potato Genotypes. Front Plant Sci 11:169. DOI: 10.3389/fpls.2020.00169.

Dufourc E.J. (2008) The role of phytosterols in plant adaptation to temperature. Plant Signal Behav 3:133-4. DOI: 10.4161/psb.3.2.5051.

Engel K.M., Prabutzki P., Leopold J., Nimptsch A., Lemmnitzer K., Vos D.R.N., Hopf C., Schiller J. (2022) A new update of MALDI-TOF mass spectrometry in lipid research. Prog Lipid Res 86:101145. DOI: 10.1016/j.plipres.2021.101145.

Feng S., Wang L., Shao P., Lu B., Chen Y., Sun P. (2022) Simultaneous analysis of free phytosterols and phytosterol glycosides in rice bran by SPE/GC-MS. Food Chem 387:132742. DOI: 10.1016/j.foodchem.2022.132742.

Francesca S., Vitale L., Arena C., Raimondi G., Olivieri F., Cirillo V., Paradiso A., Pinto M.C., Maggio A., Barone A., Rigano M.M., Luo Z.B. (2021) The efficient physiological strategy of a novel tomato genotype to adapt to chronic combined water and heat stress. Plant Biology 24:62-74. DOI: 10.1111/plb.13339.

Gomes do Ó L.M., Cova A.M.W., Neto A.D.d.A., da Silva N.D., Silva P.C.C., Santos A.L., Gheyi H.R., da Silva L.L. (2022) Osmotic adjustment, production, and post-harvest quality of mini watermelon genotypes differing in salt tolerance. Scientia Horticulturae 306. DOI: 10.1016/j.scienta.2022.111463.

Guo R., Hao W., Gong D. (2012) Effects of Water Stress on Germination and Growth of Linseed Seedlings (*Linum usitatissimum* L.), Photosynthetic Efficiency and Accumulation of Metabolites. Journal of Agricultural Science 4. DOI: 10.5539/jas.v4n10p253.

Hajihashemi S., Sofo A. (2018) The effect of polyethylene glycol-induced drought stress on photosynthesis, carbohydrates and cell membrane in Stevia rebaudiana grown in greenhouse. Acta Physiologiae Plantarum 40. DOI: 10.1007/s11738-018-2722-8.

Hasanuzzaman M., Nahar K., Gill S.S., Fujita M. (2013) Drought stress responses in plants, oxidative stress, and antioxidant defense. Climate change and plant abiotic stress tolerance:209-250.

Hodges D.M., DeLong J.M., Forney C.F., Prange R.K. (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604-611. DOI: 10.1007/s004250050524.

Hossain M.A., Wani S.H., Bhattacharjee S., Burritt D.J., Tran L.-S.P. (2016) Drought Stress Tolerance in Plants, Vol 1 Springer.

Hou Q., Ufer G., Bartels D. (2016) Lipid signalling in plant responses to abiotic stress. Plant Cell Environ 39:1029-48. DOI: 10.1111/pce.12666.

Huang Y., Bie Z., He S., Hua B., Zhen A., Liu Z. (2010) Improving cucumber tolerance to major nutrients induced salinity by grafting onto *Cucurbita ficifolia*. Environmental and Experimental Botany 69:32-38. DOI: https://doi.org/10.1016/j.envexpbot.2010.02.002.

Kubota C. (2016) Growth, Development, Transpiration and Translocation as Affected by Abiotic Environmental Factors, Plant Factory, Elsevier. pp. 151-164.

Kuczynska A., Cardenia V., Ogrodowicz P., Kempa M., Rodriguez-Estrada M.T., Mikolajczak K. (2019) Effects of multiple abiotic stresses on lipids and sterols profile in barley leaves (*Hordeum vulgare* L.). Plant Physiol Biochem 141:215-224. DOI: 10.1016/j.plaphy.2019.05.033.

Li H., Yang H., Ma C., Zhang W., Xu Q., Chen M., Ma Y. (2022) Determination of three oryzanols in rice by mixed-mode solid-phase extraction coupled with high performance liquid chromatography-tandem mass spectrometry. Se Pu 40:746-752. DOI: 10.3724/SP.J.1123.2021.12016.

Magana Ugarte R., Escudero A., Gavilan R.G. (2019) Metabolic and physiological responses of Mediterranean high-mountain and alpine plants to combined abiotic stresses. Physiol Plant 165:403-412. DOI: 10.1111/ppl.12898.

Maharjan P., Jacobs J.L., Deighton M.H., Panozzo J.F. (2018) A high-throughput method using Ultra-Performance Liquid Chromatography to determine water-soluble carbohydrate concentrations in pasture plants. Grass and Forage Science 73:562-571. DOI: 10.1111/gfs.12315.

Mamode Cassim A., Gouguet P., Gronnier J., Laurent N., Germain V., Grison M., Boutte Y., Gerbeau-Pissot P., Simon-Plas F., Mongrand S. (2019) Plant lipids: Key players of plasma membrane organization and function. Prog Lipid Res 73:1-27. DOI: 10.1016/j.plipres.2018.11.002.

Mandizvo T., Odindo A.O., Mashilo J. (2021) Citron Watermelon Potential to Improve Crop Diversification and Reduce Negative Impacts of Climate Change. Sustainability 13. DOI: 10.3390/su13042269.

Mandizvo T., Odindo A.O., Mashilo J., Magwaza L.S. (2022a) Drought tolerance assessment of citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.) accessions based on morphological and physiological traits. Plant Physiology and Biochemistry 180:106-123. DOI: 10.1016/j.plaphy.2022.03.037.

Mandizvo T., Odindo A.O., Mashilo J., Sibiya J., Beck-Pay S.L. (2022b) Phenotypic Variability of Root System Architecture Traits for Drought Tolerance among Accessions of Citron Watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey). Plants 11. DOI: 10.3390/plants11192522.

Mashilo J., Shimelis H., Ngwepe R.M. (2021) Genetic resources of bottle gourd (*Lagenaria siceraria* (Molina) Standl.] and citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.): implications for genetic improvement, product development and commercialization: a review. South African Journal of Botany. DOI: 10.1016/j.sajb.2021.10.013.

Mashilo J., Shimelis H., Odindo A.O., Amelework B. (2017) Genetic diversity and differentiation in citron watermelon [*Citrullus lanatus* var. *citroides*] landraces assessed by simple sequence repeat markers. Scientia Horticulturae 214:99-106. DOI: 10.1016/j.scienta.2016.11.015.

Mathur S., Agrawal D., Jajoo A. (2014) Photosynthesis: response to high temperature stress. J Photochem Photobiol B 137:116-26. DOI: 10.1016/j.jphotobiol.2014.01.010.

McCready R.M., Guggolz J., Silviera V., Owens H.S. (2002) Determination of Starch and Amylose in Vegetables. Analytical Chemistry 22:1156-1158. DOI: 10.1021/ac60045a016.

McElroy J.S., Kopsell D.A. (2009) Physiological role of carotenoids and other antioxidants in plants and application to turfgrass stress management. New Zealand Journal of Crop and Horticultural Science 37:327-333. DOI: 10.1080/01140671.2009.9687587.

Meena S.K., Pandey R., Sharma S., Gayacharan, Vengavasi K., Dikshit H.K., Siddique K.H.M., Singh M.P. (2021) Cross tolerance to phosphorus deficiency and drought stress in mungbean is regulated by improved antioxidant capacity, biological N2-fixation, and differential transcript accumulation. Plant and Soil 466:337-356. DOI: 10.1007/s11104-021-05062-0.

Meher P., Shivakrishna P., Ashok Reddy K., Manohar Rao D. (2018) Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi J Biol Sci 25:285-289. DOI: 10.1016/j.sjbs.2017.04.008.

Mittler R. (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15-9. DOI: 10.1016/j.tplants.2005.11.002.

Ngwepe R.M., Mashilo J., Shimelis H. (2019) Progress in genetic improvement of citron watermelon (Citrullus lanatus var. citroides): a review. Genetic Resources and Crop Evolution 66:735-758. DOI: 10.1007/s10722-018-0724-4.

Panjekobi M., Einali A. (2021) Trehalose treatment alters carbon partitioning and reduces the accumulation of individual metabolites but does not affect salt tolerance in the green microalga Dunaliella bardawil. Physiology and Molecular Biology of Plants. DOI: 10.1007/s12298-021-01078-z.

Qin J., Bian C., Liu J., Zhang J., Jin L. (2019) An efficient greenhouse method to screen potato genotypes for drought tolerance. Scientia Horticulturae 253:61-69. DOI: 10.1016/j.scienta.2019.04.017.

Rivero R.M., Ruiz J.M., García P.C., López-Lefebre L.R., Sánchez E., Romero L. (2001) Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. Plant Science 160:315-321. DOI: 10.1016/s0168-9452(00)00395-2.

Roche Y., Gerbeau-Pissot P., Buhot B., Thomas D., Bonneau L., Gresti J., Mongrand S., Perrier-Cornet J.M., Simon-Plas F. (2008) Depletion of phytosterols from the plant plasma membrane provides evidence for disruption of lipid rafts. FASEB J 22:3980-91. DOI: 10.1096/fj.08-111070.

Rogowska A., Szakiel A. (2020) The role of sterols in plant response to abiotic stress. Phytochemistry Reviews 19:1525-1538. DOI: 10.1007/s11101-020-09708-2.

Rupasinghe T.W.T., Roessner U. (2018) Extraction of Plant Lipids for LC-MS-Based Untargeted Plant Lipidomics. Methods Mol Biol 1778:125-135. DOI: 10.1007/978-1-4939-7819-9_9.

Santos G.L.D., Pereira F.H.F., Sousa V.F.D.E.O., Suassuna C.D.F., Santos A.P.D.L., Barros JÚNior A.P. (2022) Cytokinin and Auxin Influence on Growth and Quality of Watermelon Irrigated with Saline Water. Revista Caatinga 35:677-685. DOI: 10.1590/1983-21252022v35n319rc.

Saroj P.L., Choudhary B.R. (2020) Improvement in cucurbits for drought and heat stress tolerance — a review. Current Horticulture 8. DOI: 10.5958/2455-7560.2020.00013.8.

Schlag S., Gotz S., Ruttler F., Schmockel S.M., Vetter W. (2022) Quantitation of 20 Phytosterols in 34 Different Accessions of Quinoa (*Chenopodium quinoa*). J Agric Food Chem 70:9856-9864. DOI: 10.1021/acs.jafc.2c03374.

Schwarz D., Rouphael Y., Colla G., Venema J.H. (2010) Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. Scientia Horticulturae 127:162-171. DOI: 10.1016/j.scienta.2010.09.016.

Shuai X., Dai T., Chen M., Liu C.-m., Ruan R., Liu Y., Chen J. (2022) Characterization of lipid compositions, minor components and antioxidant capacities in macadamia (*Macadamia integrifolia*) oil from four major areas in China. Food Bioscience 50. DOI: 10.1016/j.fbio.2022.102009.

Tan S., Niu Y., Liu L., Su A., Hu C., Meng Y. (2019) Development of a GC-MS/SIM method for the determination of phytosteryl esters. Food Chem 281:236-241. DOI: 10.1016/j.foodchem.2018.12.092.

Turakainen M., Hartikainen H., Seppanen M.M. (2004) Effects of selenium treatments on potato (Solanum tuberosum L.) growth and concentrations of soluble sugars and starch. J Agric Food Chem 52:5378-82. DOI: 10.1021/jf040077x.

Uarrota V.G., Stefen D.L.V., Leolato L.S., Gindri D.M., Nerling D. (2018) Revisiting Carotenoids and Their Role in Plant Stress Responses: From Biosynthesis to Plant Signaling Mechanisms During Stress, in: D. K. Gupta, et al. (Eds.), Antioxidants and Antioxidant Enzymes in Higher Plants, Springer International Publishing, Cham. pp. 207-232.

Ul Hassan M., Rasool T., Iqbal C., Arshad A., Abrar M., Abrar M.M., Habib-ur-Rahman M., Noor M.A., Sher A., Fahad S. (2021) Linking Plants Functioning to Adaptive Responses Under Heat Stress Conditions: A Mechanistic Review. Journal of Plant Growth Regulation 41:2596-2613. DOI: 10.1007/s00344-021-10493-1.

Valitova J.N., Sulkarnayeva A.G., Minibayeva F.V. (2016) Plant Sterols: Diversity, Biosynthesis, and Physiological Functions. Biochemistry (Mosc) 81:819-34. DOI: 10.1134/S0006297916080046.

Wahid A., Gelani S., Ashraf M., Foolad M.R. (2007) Heat tolerance in plants: An overview. Environmental and Experimental Botany 61:199-223. DOI: https://doi.org/10.1016/j.envexpbot.2007.05.011.

Yang Y., Wang L., Tian J., Li J., Sun J., He L., Guo S., Tezuka T. (2012) Proteomic study participating the enhancement of growth and salt tolerance of bottle gourd rootstock-grafted watermelon seedlings. Plant Physiology and Biochemistry 58:54-65. DOI: https://doi.org/10.1016/j.plaphy.2012.05.026.

Yavuz D., Seymen M., Süheri S., Yavuz N., Türkmen Ö., Kurtar E.S. (2020) How do rootstocks of citron watermelon (*Citrullus lanatus* var. *citroides*) affect the yield and quality of watermelon under deficit irrigation? Agricultural Water Management 241. DOI: 10.1016/j.agwat.2020.106351.

Yavuz N. (2021) Can grafting affect yield and water use efficiency of melon under different irrigation depths in a semi-arid zone? Arabian Journal of Geosciences 14. DOI: 10.1007/s12517-021-07498-1.

Yonny M.E., Rodriguez Torresi A., Cuyamendous C., Reversat G., Oger C., Galano J.M., Durand T., Vigor C., Nazareno M.A. (2016) Thermal Stress in Melon Plants: Phytoprostanes and Phytofurans as Oxidative Stress Biomarkers and the Effect of Antioxidant Supplementation. J Agric Food Chem 64:8296-8304. DOI: 10.1021/acs.jafc.6b03011.

Zandalinas S.I., Balfagon D., Gomez-Cadenas A., Mittler R. (2022) Plant responses to climate change: metabolic changes under combined abiotic stresses. J Exp Bot 73:3339-3354. DOI: 10.1093/jxb/erac073.

Zhao C., Zhang H., Song C., Zhu J.K., Shabala S. (2020) Mechanisms of Plant Responses and Adaptation to Soil Salinity. Innovation (Camb) 1:100017. DOI: 10.1016/j.xinn.2020.100017.

Chapter 7: Summary and conclusion

7.1 General discussion

The background of this study is based on the potential of citron watermelon to improve crop diversification and reduce negative impacts of climate change (crop failure and food insecurity). Despite being less photo-efficient, especially under water stress, citron watermelon has mechanisms to tolerate, escape and avoid drought (Mandizvo et al., 2021). Under the context of climate change and crop production, citron watermelon is an interesting plant species whose capacity to tolerate adverse environmental conditions (water stress) and remarkable nutritional qualities warrant research in plant breeding, agronomy, and ecology. Citron watermelon, exhibit a broad gene pool (Mashilo et al., 2021; Ngwepe et al., 2019) that could be exploited to improve (i) key yield-influencing attributes under biotic and abiotic stress and (ii) nutritional quality.

Seed coat colour is an important phenotypic trait to consider when selecting citron watermelon for roasting and processing other value-added products (Mandizvo and Odindo, 2019; Ngwepe et al., 2021; Qutob et al., 2008). Chapter 3 (1st objective) determined whether physical characteristics, biochemical and nutrient compositions in mature seeds of genetically distinctive genotypes of citron watermelon with varied seed coat colours differ; and (ii) it tested whether seed physical characteristics (coat colour) and biochemical compositions (cellulose and lignin content) are associated with citron watermelon nutritive quality, palatability (roasting, taste and ease of chewing) and popping yield. Brown and red-coloured seeds have a higher popping yield than dark-coloured seeds with poor popping ability and are prone to burning during roasting. The popping ability of the seed was associated with increased calcium concentration. In contrast, seed coat thickness was closely related to hemicellulose contents and cellulose across all seed coat colours. Findings of chapter 3 will guide breeding strategies to develop nutritionally improved genotypes of citron watermelon for food and feed and developing value-added nutraceuticals.

The second objective (chapter 4) revealed variation in drought tolerance among South African citron watermelon landrace accessions. Forty citron watermelon accessions evaluated in showed varying levels of drought tolerance based on morphological (leaf area and root traits), physiological traits (leaf gas exchange and chlorophyll fluorescence) and agronomic traits (fruit yield). These allowed five distinct groupings namely: A (highly drought-tolerant), B (drought-tolerant), C (moderately drought tolerant), D (drought-sensitive) and E (highly drought-sensitive) based on various drought tolerance indices. The following accessions (WWM02,

WWM-05, WWM-09, WWM-15, WWM-37(2), WWM-39, WWM-41 (A), WWM-46, WWM-47, WWM-57, WWM-64, WWM-66, WWM-68 and WWM-79) were categorized as highly-drought tolerant and accessions WWM-03, WWM-08, WWM-14, WWM-21, WWM-33, WWM-35(1), WWM-35(2), WWM-67 and WWM-76 as drought tolerant. These are useful genetic stock for genetic improvement of drought tolerance in this crop and related cucurbit crop including sweet watermelon.

Understanding root system development under drought conditions in this species could aid in breeding high-yielding, and improved cultivars with enhanced water-use and drought tolerance traits adapted to dry conditions (Farooq et al., 2009; Monneveux et al., 2013; Pandey and Shukla, 2016). The third objective explored the root system architecture of citron watermelon and identified drought-adaptive root traits for cultivar improvement under water-stressed environments. This study showed that plasticity and biomass allocation shift in different ways according to genotype to optimise the use of limited resources. The study found significant phenotypic variation in root architecture among citron watermelon accessions that may relate to differences in water uptake. The following RSA traits, including total root length, root system width, convex hull area and total root volume, were associated with drought tolerance.

7.2 Conclusion and recommendations

Drought is a major constraint that impairs crop yield. To facilitate drought tolerance and maintaining yield under such calamity, a thorough understanding of various physiological traits that govern the yield under water stress condition and integration of tolerance strategies from different disciplines is a prerequisite. Roots are critical in extracting water from soil and their architecture and anatomical traits largely determines crop functioning under water related stress. Various root parameters like deep root with greater xylem vessel radii and lower resistance to water flux can be used as a criterion for selecting drought tolerant genotypes. Physiological factors like water use efficiency (*WUE*) and transpiration efficiency (*TE*) are associated with increased yield under drought. Breeding for improved *WUE*, *TE* and various root traits could be beneficial for improving citron watermelon yield under drought stress. The study focused on architectural phenes, it is recommended that future research must explore; (i) anatomical phenes (more cortical aerenchyma, reduced cortical cell size, reduced cortical cell number and suberization of outer cortical cell) and (ii) metabolic phenes (greater exudation of biological nitrification inhibitors, greater exudation of H^+ and organic compounds, greater phosphate uptake capacity of root cell and greater N uptake capacity of root cells).

While our physiology findings improve understanding of complex network of drought tolerance-related traits, future research must focus on molecular biology and genomic approaches to identify candidate genes and quantitative trait loci (QTLs) associated with these traits. The QTLs for morpho-physiological and yield traits under drought stress in citron South African citron watermelon germplasm have not been identified through molecular mapping approaches and are highly relevant for the selection of drought-resistant genotypes. New technology such as CRISPR/Cas9 can be used to edit genes in citron watermelon that confer drought stress. Understanding the physiological traits of plants under drought and then incorporation of these improved drought-associated-traits through physiological breeding might be helpful in sustaining citron watermelon yield and productivity.

References

Farooq M., Wahid A., Kobayashi N., Fujita D., Basra S.M.A. (2009) Plant Drought Stress: Effects, Mechanisms and Management, in: E. Lichtfouse, et al. (Eds.), Sustainable Agriculture, Springer Netherlands, Dordrecht. pp. 153-188.

Mandizvo T., Odindo A.O. (2019) Seed coat structural and imbibitional characteristics of dark and light coloured Bambara groundnut (*Vigna subterranea* L.) landraces. Heliyon 5:e01249. DOI: 10.1016/j.heliyon.2019.e01249.

Mandizvo T., Odindo A.O., Mashilo J. (2021) Citron Watermelon Potential to Improve Crop Diversification and Reduce Negative Impacts of Climate Change. Sustainability 13. DOI: 10.3390/su13042269.

Mashilo J., Shimelis H., Ngwepe R.M. (2021) Genetic resources of bottle gourd (*Lagenaria siceraria* (Molina) Standl.] and citron watermelon (*Citrullus lanatus* var. *citroides* (L.H. Bailey) Mansf. ex Greb.): implications for genetic improvement, product development and commercialization: a review. South African Journal of Botany. DOI: 10.1016/j.sajb.2021.10.013.

Monneveux P., Ramirez D.A., Pino M.T. (2013) Drought tolerance in potato (*S. tuberosum* L.): Can we learn from drought tolerance research in cereals? Plant Sci 205-206:76-86. DOI: 10.1016/j.plantsci.2013.01.011.

Ngwepe R.M., Mashilo J., Shimelis H. (2019) Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides*): a review. Genetic Resources and Crop Evolution 66:735-758. DOI: 10.1007/s10722-018-0724-4.

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

Pandey V., Shukla A. (2016) Improving Crop Yield Under Drought Stress Through Physiological Breeding, in: M. A. Hossain, et al. (Eds.), Drought Stress Tolerance in Plants, Vol 1: Physiology and Biochemistry, Springer International Publishing, Cham. pp. 331-348.

Qutob D., Ma F., Peterson C.A., Bernards M.A., Gijzen M. (2008) Structural and permeability properties of the soybean seed coat. Botany 86:219-227. DOI: 10.1139/b08-002.