IMPACTS OF DROUGHT STRESS ON COFFEE (*Coffea arabica* L.) GENOTYPES: INSIGHTS FROM GERMINATION, GROWTH, PHYSIOLOGICAL, AND BIOCHEMICAL RESPONSES

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Impacts of Drought Stress on Coffee (*Coffea arabica* L.) Genotypes: Insights from Germination, Growth, Physiological, and Biochemical Responses

By

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SUMMARY

Impacts of Drought Stress on Coffee (*Coffea arabica* L.) Genotypes: Insights from Germination, Growth, Physiological, and Biochemical Responses

Habtamu Chekol Fantahun, **PhD Dissertation**

Addis Ababa University, **2024**

Drought stress is one of the major abiotic factors affecting crop growth and limiting production worldwide. Differences in drought tolerance among *Coffea arabica* genotypes have been observed, particularly in Ethiopia, where the effects of drought stress on coffee growth and yield have been documented. However, a comprehensive understanding of the morphological, physiological, and molecular processes in Ethiopian Arabica coffee genotypes under drought stress during germination, seedling, and adult development remains limited. This study delves into investigating the germination, growth, physiology, and molecular performance of three distinct groups of coffee genotypes categorized as relatively tolerant (*Ca*74140, *Ca*74112, and *Ca*74110), moderately sensitive (*Ca*74158, *Ca*74165, and *Ca*J-21), and sensitive (*Ca*754, *Ca*J-19, and *Ca*Geisha), behaving differently in their physiological response to a dry soil condition. Therefore, a poly-propagator experiment under a shade house was conducted to investigate the impact of drought stress on the germination potential of the genotypes. Greenhouse measurements were then performed to evaluate the effects of drought stress on seedling development, encompassing growth, physiology, and molecular responses across coffee genotypes. Results indicated distinctive responses among coffee genotypes during germination, with relatively tolerant genotypes exhibiting swifter and more comprehensive germination, correlated with higher moisture content (Mc), greater seed surface area to volume ratio (SA/SV),

and elevated coefficients of velocity and variation of germination (CVG, CV_t), and germination index (GI). Furthermore, relatively tolerant genotypes displayed enhanced seedling vigor (VI). Under drought stress conditions, leaf, stem, and root development, leaf relative water content (RWC), water potential (Ψ), stomatal conductance (Gs), net assimilation rate (A_{net}), and transpiration rate (E) significantly declined across all genotypes. Nevertheless, relatively tolerant genotypes demonstrated better resilience compared to other groups. Metabolite analysis unveiled distinctive accumulation patterns, notably higher levels of certain compounds like glucose, maltose, tryptophan, L-cysteine, malic acid, oxalic acid, pyruvic acid, and shikimic acids, in relatively drought-tolerant genotypes, while other metabolites showed decreased accumulation. Osmotic adjustment via compatible solutes and energy-associated metabolites emerged as a significant mechanism associated with growth and physiological responses, emphasizing the role of osmotic potential modulation in drought tolerance among coffee genotypes. This study underscores the diverse tolerance levels to drought among Ethiopian coffee genotypes. Traits such as rapid and complete germination, higher growth, relative water content, water potential, gaseous exchange, and osmotic adjustment signify heightened tolerance to drought episodes. These attributes could serve as useful markers for identifying drought-tolerant genotypes in plant biotechnology. Overall, this study highlights the pivotal role of seed traits, germination, and post-germination events, encompassing growth, physiological responses, and metabolite dynamics, in delineating drought tolerance among coffee genotypes.

Key words: *Coffea arabica*, drought stress, seed germination, water potential, gas exchange, metabolites alteration

DEDICATION

I dedicate my dissertation work to my families, who have been a source of patience, motivation, support, strength, and love for me throughout the Ph.D. period. I also dedicate this dissertation to my friends and colleagues who have encouraged and supported me throughout the process of the Ph.D. study. Finally, I dedicate this dissertation to the coffee farmers of Ethiopia, who are sacrificing their livelihoods and facing many challenges in the production of coffee beans and who needs to cultivate drought-tolerant coffee genotypes and develop more resilient production systems.

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Habtamu Chekol Fantahun

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LIST OF ACRONYMS

A_{net}	Net Carbon Assimilation
Ca	*Coffea arabica* L.
CMS	Cell Membrane Stability
CVG	Coefficient of the Velocity of Germination
CV_t	Coefficient of Variation of Germination Time
DADB	Days after Drought Begins
DMC	Dry Matter Content
E	Transpiration Rate
GABA	γ-Aminobutyric Acid
GC–MS	Gas Chromatography–Mass Spectrometry
GI	Germination Index
GP	Germination Percentage
GRI	Germination Rate Index
Gs	Stomatal Conductance
Gv	Germination Value
LA	Leaf Area
LAR	Leaf Area Ratio
LDM	Leaf Dry Mass
LFM	Leaf Fresh Mass
LMF	Leaf Mass Fraction
LN	Leaf Number
MDG	Mean Daily Germination
MGT	Mean Germination Time
PCA	Principal Component Analyses
Pv	Peak Value For Germination
R/S^r	Root to Shoot Ratio
RCI	Relative Cell Injury
RDM	Root Dry Mass
RFM	Root Fresh Mass
RL	Root Length

RMF	Root Mass Fraction
RN	Root Number
RV	Root Volume
RWC	Relative Water Content
SD	Stem Collar Diameter
SDM	Stem Dry Mass
SFM	Stem Fresh Mass
SH	Stem Height
SLA	Specific Leaf Area
SMF	Stems Mass Fraction
SRL	Specific Root Length
SSL	Specific Stem Length
TCA	Tricarboxylic Acid Cycle
TDM	Total Dry Mass
TFM	Total Fresh Mass
TL	Time Line
LTW	Leaf Turgid Weight
U	Uncertainty of Germination Process
Z	Synchrony of Germination Process

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1. CHAPTER ONE

General Introduction

1.1. Introduction

1.1.1. Coffee: origin and distribution

Next to oil, coffee is one of the most exchangeable worldwide commodities and is the source of income for approximately 80 developing countries (ICO, 2022). The origin and natural habitat of all Coffee species is the understory of African tropical forests, probably the highlands of Ethiopia, in the southwest region of Ethiopia, in a site known as Kaffa (Inga *et al*., 2003). Coffee was taken From Ethiopia to Arabia as early as 575 AD and introduced to Europe, first in Italy and later in England around 1710 (Aude, 2004). Coffee reached America in 1718, but an important milestone was achieved in 1727 with the planting of coffee in Brazil, which is now the world's dominant coffee producer (Stellmacher and Grote, 2011). In 1893, not far from its place of origin in Ethiopia; Kenya and Tanzania introduced coffee from Brazil, 600 years of transcontinental journey (Haarer, 1958; Jose, 2012).

Coffee is currently grown in tropical and subtropical regions of Ethiopia, Kenya, Tanzania, Uganda, India, Thailand, Indonesia, Vietnam, Brazil, Jamaica, Cuba, and Puerto Rico, the Hawaiian Islands (Smith, 1985; Davis *et al*., 2019). *C. arabica* occurs in the montane rainforest of Ethiopia, where it is considered the center of origin and genetic diversity (Arega Zeru, 2006). The major coffee-producing areas in Ethiopia are located in the areas of Sidama, Gedeo, Harerge, Jimma, Lekemit, Wellega, Sheka-Kefa, Bench-Maji, Yayu, Limu, Tepi, Bebeka, and others (Tadesse Woldemariam and Feyera Senbeta, 2008).

1.1.2. Coffee: taxonomy and production

Coffee trees are tropical woody plants of the Rubiaceae and are classified into two genera. These are the genus *Coffea* L. and *Psilanthus* Hook f. (Inga *et al*., 2003). The genus *Coffea*, which is economically important, consists of approximately 100 species (Chaparro *et al*., 2004). The genus

contains the three most important species used in the production of the beverage coffee, *viz*. *Coffea arabica* (arabica coffee), *Coffea canephora* (robusta coffee), and *Coffea liberica* (Liberian, Liberica, or excelsa coffee) (Anthony *et al*., 2001; Arega Zeru, 2006). Of these, *C. arabica* is by far the most important commercial species and is considered a source of superior coffee, contributing to over 70% of the world's coffee production (Inga *et al*., 2003; Esayas Aga *et al*., 2003; Elmar and Jean-Francois, 2006).

Arabica coffees are characterized as evergreen, shrubs or small trees, growing up to 5 m tall. Usually, the shoot has an orthotropic (vertical) main stem and plagiotropic (horizontal) branches (Campanha *et al*., 2005). Auxiliary buds (buds arranged around an axle, i.e., stem or branch) on the stem consist of a primary bud that can grow into a lateral branch and several buds that develop into suckers and inflorescences (that is where the flowers grow from). The primary branches could give rise to secondary and tertiary (DaMatta *et al*., 2008). *Arabica's* leaves are dark green, opposite, elliptical, short-petioled, glossy, 2-8 cm broad, 5-20 cm long (Tavares-Junior *et al*., 2002); flowers white, in axillary clusters, corolla tubular, fragrant, 1 cm long, 5-lobed; small calyx, cup-shaped and opening 8-12 days after wetting (DaMatta *et al*., 2008); fruit a drupe, oval-elliptic, green when immature, red when ripening, black upon drying, about 1.5 cm long, 7-9 months to maturity (Ronchi *et al*., 2006); seeds ellipsoidal, usually 2, 8-13 mm long, inner surface deeply grooved, consisting green corneous endosperm, and small embryo (Vaast *et al*., 2005); the roots system initially enters 30-50 cm layer, and when mature extends about 1.50 m in diameter from the stem and penetrates 2 to 4 m in length. The root system is highly plastic and its distribution and length are age-dependent (Catalina *et al*., 2010). Besides, the coffee root system varies with planting density, genotypes, soil characteristics, cultural practices, and weed competition (Huxley,

1964). *C. arabica* is allotetraploid (*2n=4x*=44) and is completely self-fertile (Anthony *et al.*, 2001; dos Santos and Mazzafera, 2013).

The 2020 and 2021 data on the global average production of coffee beans was around 130 million 60-kg bags per year, of which Arabica coffee production amounted to 100 million 60-kg bags (ICO, 2022). Brazil covers the majority of Arabica coffee, making up about 44% of global production, and Ethiopia (5%), Guatemala (5%), Peru (5%), and Honduras (4%) (FAOSTAT, 2021). Ethiopia is the first Arabica coffee producer in Africa and the tenth-largest exporter worldwide. The average annual production amounts to about ~471,000 tons (ECX, 2021), and the average yield is about 0.71 tons/ha (CSA, 2021). Ethiopian coffee is intrinsically organic and is renowned for its superior quality (Samuel and Eva, 2008; Taye Kufa, 2012). Smallholder farmer's account for more than 95% of the total coffee produced in Ethiopia, but the farming systems are still traditional (Taye Bekele, 2011).

1.1.3. Coffee: ecology

Rainfall requirements depend on the retention properties of the soil, atmospheric humidity and cloud cover, as well as cultivation practices. The optimum annual rainfall range is 1200-1800 mm for *C. arabica* (Tesfaye Shimber, 2018[a]). Abundant rainfall throughout the year is often responsible for scattered harvest and low yields. Lack of a dry period can also limit coffee cultivation in lowland tropical regions (Maestri and Barros, 1977). Precipitation in excess of 2500 to 3000 mm begins to be detrimental (Aude, 2004).

The optimum mean annual temperature range for *C. arabica* is 18-21 ºC (Vaast *et al.*, 2005). Above 23 ºC, development and ripening of fruits are accelerated, often leading to loss of quality (DaMatta, 2004). Relatively high temperature (above 25 °C) during blossoming, especially if associated with a prolonged dry season, may cause abortion of flowers and growth impairment

(Tavares-Junior *et al*., 2002). It should be noted, however, that selected cultivars under intensive management conditions have allowed *C. arabica* plantations to be spread to marginal regions with average temperatures as high as 24-25 ºC, with satisfactory yields (DaMatta and Ramalho, 2006). On the other hand, in regions with a mean annual temperature below 17 ºC, growth is largely depressed. Occurrence of frosts, even if sporadic, may strongly limit the economic success of the crop and leaves (DaMatta *et al*., 2008).

Air humidity has a significant impact on the vegetative growth of the coffee tree. Humidity plays a role in governing the loss of water or moisture by evapo-transpiration. When it is high, loss of water is reduced and vice versa. Especially it is important during the dry season as high humidity decreases the stress on the coffee trees thereby extending the rainless period through which the plants will survive without damage (Ronchi *et al*., 2006). Arabica coffee successfully grows under less humid atmosphere, comparable to that of the Ethiopian highlands (Haarer, 1958; Coste, 1992). Wind may have different effects on the growth and yield of Arabica coffee. In coffee plantations subjected to large wind shears and advection, crop yield is usually depressed. Wind stress may lead to a reduction of leaf area and internode length of the orthotropic and plagiotropic branches, in addition to severely damaging leaves and buds and exacerbating shedding of developing flowers and fruits. Hot winds increase crop evapotranspiration and therefore the rainfall (or irrigation) requirements of the trees increase. Where strong wind is frequent, windbreaks or shelter trees are to be recommended as both may improve crop performance (Chaparro *et al*., 2004).

Coffee has evolved as a shade adapted species, because it is native to the forested Ethiopian highlands (Taye Kufa, 2012). The photosynthetic rate is more efficient in shade leaves. In their native habitats, arabica coffee produces few flowers, as floral initiation is light dependent, and

this limits the amount of fruits that a tree can produce (Rodrigues *et al*., 2021). In high light intensities, arabica coffee trees produce greater number of flowers and thus cherries (Samuel Gebresselasei and Eva, 2008). As coffee cannot shed excess fruit, the tree becomes committed to filling these coffee beans, requiring inputs such as minerals and nutrients greater than can be sourced (Taye Bekele, 2011).

Although Arabica coffee tolerates soil pH from 4 to 8, pH of 5.2 to 6.2 is preferred. Good drainage is essential, and soil textures lighter than clays are best (Wrigley, 1995). In fact it grows well in the clay-silcaceous soils of granite as it does on soils of volcanic origin with diverse characteristics or even on alluvial soils (Taye Kufa, 2006). Water holding capacity and depth are the other two properties to be considered. Since it provides sufficient available water, higher water holding capacity helps to maintain evapo-transpiration during dry season, while deep soils allow root proliferation by offering a larger volume of soil which contains more water and nutrients around the coffee trees (Vaast *et al*., 2005). Deep soils are especially necessary in areas where there is a long dry season coupled with lower rainfall. Arabica coffee can grow well on deep soil. Soils with high organic matter and also available phosphorus (which is essential for shoot growth and leaf initiation) are highly suitable (DaMatta *et al*., 2008).

1.1.4. Coffee: cultivation and management

Coffee propagation is done by seed but also budding, grafting, and cuttings have also been used as tools for the propagation of the species (WCR, 2021). In Ethiopia, a more successful method is propagation using seeds, and when the seedlings reach 6-12 months, depending on the genotypes, they are taken to fields, hardened, and then planted under the shade of native trees such as *Acacia abyssinica*, *Cordia africana*, *Millettia ferruginea*, *Erythrina brucei*, *Albizia*

schimperiana, *Ficus vasta*, and *Ficus sur* (Adugna and Paul, 2011; Taye Kufa, 2012; Mayoli and Gitau, 2012; Habtamu Chekol, 2013; Tesfaye Shimber, 2018[a]).

Pruning is a common practice to increase the lateral branches and berry formation. Shading tends to favor leaf and shoot growth at the expense of root growth. Shading may be useful when plants are young, but later unduly shady conditions may reduce yields, especially when the trees are mature and other environmental factors such as moisture, temperature, and soil fertility are favorable (Coste, 1992; Catalina *et al*., 2010; Habtamu Chekol, 2013). Trees come into bearing 3-4 years after planting and are in full bearing at 6-8 years. Fruits mature 7-9 months after flowering (DaMatta *et al*., 2007). Selective picking of ripe, red fruits produces the highest quality coffee beans. The crop ripens over a period of several weeks (Vaast *et al*., 2005). In Brazil, all berries are stripped onto the ground on clothes at almost the same time, usually in April to June of the year (Ronchi *et al*., 2006).

In Ethiopia, the harvest season is from October to December after the long rainy season. Berries are dried in the sun, but artificial heat is used in some humid regions. After picking, de-pulping of berries is increasingly practiced (Taye Kufa, 2012). Coffee is used as a source of stimulant (caffeine), for the preparation of fermented drinks from the pulp, a flavoring agent in ice cream, pastries, candies, and liqueurs preparation, and a source of manure, and fed to cattle (Coste, 1992; Barone and Roberts, 1996; Alan, 2014). In Ethiopia, coffee cultivation takes place under four broad production systems, i.e., forest coffee (8-10%), semi-forest coffee (30-35%), garden coffee (50-57%), and modern coffee plantations (5%) (Stellmacher and Grote, 2011; Jim and Ruth, 2014; Moat *et al.*, 2017).

1.1.5. Coffee: germination

The coffee fruit is a drupe containing two seeds, and the seed is comprised of an endosperm, embryo and spermoderm or "silver skin" (Rosa *et al*., 2010). The thickened cell walls of the endosperm are composed mainly of mannans. Seed germination "begins with the water uptake by the seed (imbibition) and ends with the elongation of the embryonic axis, usually the radicle" (Etienne *et al*., 2013). Therefore, the end of the germination process in coffee seeds corresponds with protrusion of the radicle, with expansion force of the embryo, through the endosperm (Baskin and Baskin, 2014). In a number of endosperm retaining species it has been shown that weakening of the endosperm through hydrolytic degradation of the cell walls allows the radicle to overcome endosperm resistance (Steinbrecher and Leubner-Metzger, 2017).

1.2. Background of the Study

1.2.1. Threats to coffee cultivation and production

The agronomical cultivation and industrial management of coffee crops involve some 500 million people (Gruter *et al*., 2022). In the meantime, about 25 million small farmer producers globally depend on Arabica coffee (Poltronieri and Rossi, 2016). Out of the total production, an average of 10 million tonnes, and 11 million hectares of total harvested area, Brazil covers 44%, and Ethiopia covers 5% (~471,000 tons per annum; 0.71 ton/ha) of global production (FAOSTAT, 2021).

Environmental abiotic stresses, such as drought, extreme temperature, cold, heavy metals, or high salinity, severely impair coffee growth and productivity worldwide (Joel, 2014; Davis *et al*., 2019). Among the environmental stresses, drought stresses severely impacts coffee growth, development, and production (Haggar and Schepp, 2012; Bilen *et al*., 2022). Although coffee production is strongly affected by drought events, a significant portion of the world’s coffee is cultivated in drought-prone regions where irrigation is the exception (DaMatta *et al*., 2007;

Justin *et al*., 2017). According to Moat *et al*. (2017), coffee-producing agroecological areas are facing a lack of precipitation, which is ultimately affecting coffee production in Ethiopia and its scarcity is a major deterrent to high coffee yields.

Water is essential for nutrient transport, cell growth and expansion, transpiration, photosynthesis, metabolic activities, enzymatic reactions, and others (DaMatta and Ramalho 2006; Taiz and Zieger, 2010). Various findings stated the causes of drought in the environment, differently. Dias *et al*. (2007) and Seleiman *et al*. (2021), drought stress is caused by low high and low temperatures, salinity, rainfall, and high intensity of light. Oguz *et al*. (2022) stated pseudo-physiological drought stresses as the lack of root efficiency to absorb water from the soil even if there is enough water in the soil. Bilen *et al*. (2022), drought stress occurs in plants when the transpiration rate is higher than the water uptake by roots when the soil water potential is lower than plant roots.

1.2.2. Impacts of climate change in coffee plants

Climate change is becoming more unpredictable amplifying various environmental stresses, which directly or indirectly affects the growth and development of principal crops, and the quality of their products leading to yield reduction of's yield (Seleiman *et al*., 2021). The atmospheric CO_2 concentration has increased by approximately 50% since preindustrial times to significant values currently exceeding 400 ppm (Yanez-Lopez, 2012). Over the same period, the global mean temperature has increased by 0.85 °C (Joel, 2014). By the end of this century, CO_2 concentration is predicted to rise to values as high as ∼1000 ppm in parallel with temperature increases of up to 4.8 °C (Bilen *et al*., 2022). These climate changes are also predicted to be accompanied by shifts in the frequency and severity of extreme events, including increasing heat waves, and prolonged drought episodes (Moustakas *et al*., 2022).

As a consequence of global warming, coffee plants as one of the most sensitive plants to environmental stresses are currently exposed to climate change to a greater extent, and coffee-growing geographical regions could also suffer important geographical delocalization (Justin *et al*., 2017). As a result, reports from Joel (2014) and Justin *et al*. (2017), coffee-producing countries, including Ethiopia, are facing plenty of challenges from abiotic constraints (temperature increases, elevated CO_2, precipitation decreases, drought stress, and nutrient depletion), as well as biotic constraints (e.g., weeds, insects, and diseases infestation), that directly influence the growth, development, flowering, fruit-bearing, and yield of coffee cultivation and production. Such types of stressful conditions represent significant challenges for the sustainability of coffee production on a global scale, quantitatively and qualitatively impacting harvestable coffee beans within their current production areas (Joel, 2014).

According to WCR (2021), considering the impacts of recent climate change and forecasting future tendencies in temperature and precipitation to 2050 coffee production is likely to decline by 34% in Mexico and 40% in Costa Rica, Nicaragua, and El Salvador. Thus, such loss of suitable climates leads to the migration of coffee plants towards more favorable higher altitude areas (Justin *et al*., 2017; FAOSTAT, 2021). According to Rodrigues *et al*. (2021), the main coffee producing in Brazil (Minas Gerais and São Paulo), the potential area for production would decline from 70-75% to 20-25%.

1.2.3. Impacts of drought stress

Drought stress is one of the main abiotic factors that affect all organisms. Drought occurs when soil moisture level and relative humidity in the air are low, while temperature is also high, and when the internal stem/leaf water potential is reduced and declined (Rodrigues *et al*., 2021). As a result, the extent and status of water deficit in plants are determined through tissue water potential

(Ψ_t, MPa) and relative water content (RWC, %), depending on this, the drought stress level ranges from mild (Ψ_t, ~-0.5 MPa; RWC, 70%), moderate (Ψ_t, ~-0.5 to -1.5 MPa; RWC, 60%), and severe (Ψ_t, ~<-1.5 MPa; RWC, 40%) (Taiz and Zeiger, 2010).

Drought stress in plants results either from a restricted water supply to their roots or due to an increased rate of transpiration (dos Santos and Mazzafera, 2013). Roots are the primary site of water intake in plants. The extent of force required for a plant to absorb water from the soil is known as the matric potential, and in conditions of low soil moisture, more energy is required by the plants to remove water from the soil; thus, the matric potential is greater (Seleiman *et al*., 2021).

The extent and duration of the water deprivation determines the magnitude of the stress response (Bilen *et al*., 2022). Some plants may adapt more easily than others giving them an advantage over competitors. Water stress may range from moderate, and of short duration, to extremely severe and prolonged drought (Fahad *et al*., 2017).

Growth and development are achieved through cell division, cell growth, and differentiation, and accompanied by morphological, anatomical, physiological, and molecular changes, where, in the meantime, such activities are highly vulnerable and influenced by drought stress conditions (DaMatta, 2003). Reduced turgor pressure, as a result of drought stress, limits cell division and further plant development (Seleiman *et al*., 2021). However, if the severity of drought stress continues, it may even collapse the growth and development of the whole plant system (Taiz and Zeiger, 2010). Drought stress leads to suppression of the germination process (Cavatte *et al*., 2012; Queiroz *et al*., 2019), and decreases the growth and development of leaf number and area, and root number, length, and volume, as well as facilitating leaf aging, and early maturation (DaMatta, 2003; Abreha Kibrom *et al*., 2022). According to Sharkey and Seeman, (1989) and Fahad *et al*.

(2017), drought stress disrupts the activity of osmotic balance, transpiration, stomata, photosynthesis, leaf water content, and water transmission. DaMatta, (2004), Rong-Hua *et al*. (2006), and Tardieu *et al*. (2018) also stated that drought stress inhibits the synthesis of chlorophyll and hormones, and alters primary and secondary metabolite accumulation. dos Santos and Mazzafera (2013), Cruz (2008), and Visentin *et al*. (2016) reported that drought stress affects signal transduction, transcription, and translation factors, which are later accompanied by gene expression changes and the damage-repair process.

When the severity of drought stress is high, plants generally display internal and external drought symptoms, including loss of leaf turgor, wilting, etiolation, yellowing, drooping, premature leaf downfall, thinning tree, and shrub canopy, bark and twig crack, necrosis, branch dieback, poor and stunted growth, and even death occurs (Mirian *et al*., 2006; Silva *et al*., 2022). Hence, developing and screening coffee genotypes capable of withstanding drought stress and producing high yields is therefore of utmost importance (DaMatta *et al*., 2007).

1.2.4. Responses towards drought stress

The plant's response to drought stress depends on the species, intensity, severity, and duration of the stress as well as the growth stage and genotype of the plant (Taiz and Zeiger, 2010). As a result of evolutional selection, plants usually use different adaptive mechanisms to adverse effects of drought stress (dos Santos and Mazzafera, 2013). Based on these, stress avoidance, escape, and tolerance are the three main survival strategies that plants utilize when exposed to drought stress (Dias *et al*., 2007).

During the stress avoidance strategy, plants close their stomata, hairy leaves, and cuticles develop, roots move deeper into the soil to increase water uptake, leaves roll, and consequently plants efficiently use the available water (Pamungkas *et al*., 2022). In the case of escaping

mechanisms during drought stress conditions, plants use mechanisms such as completing the vegetative cycle in a short time to pass the generative stage quickly, early flowering, self-reproduction, and seed formation (Silva *et al*., 2022).

Based on the duration of the stress, plants undergo short-, mid-, and long-term responses. During short-term responses, which occur a few seconds following the onset of water stress, plants are primarily subjected to stomatal regulation that leads to the reduction of transpiration and maximizing CO_2 intake which are responsible for the maintenance of a constant ratio of transpiration to photosynthesis (Silva *et al*., 2022). Mid-term responses are associated with the accumulation of solute, modifications in cell wall elasticity, and morphological variations (Seleiman *et al*., 2021). Long-term responses are characterized by genetic modification responsible for anatomical, morphological, and physiological modifications to balance resource utilization (Bilen *et al*., 2022).

Water is essential in the maintenance of the turgor which is essential for cell enlargement and growth, in the opening of stomata and the movements of leaves, flower petals, and various specialized plant structures (Leon-Burgos *et al*., 2022). During drought stress, due to osmotic adjustment, the accumulation of solutes in growing cells delays dehydration and allows limited growth. In plants, osmotic adjustment has the role of decreasing the osmotic potential of cells, therefore increasing the gradient for water flux inside the cell to maintain cell turgor and growth (Oguz *et al*., 2022). Depending on the genotype, organ type, and age, osmotic adjustment is variable in species (DaMatta, 2004). In plants, tolerant to drought stress, higher osmotic adjustment was found in roots than in leaves in response to water-deficit conditions (Taiz and Zeiger, 2010). Maintenance of cell turgor contributes to continued cell division and enlargement, and stomatal conductance and photosynthesis (Xiong *et al*., 2022). Therefore, any loss in turgor

pressure as a consequence of the imbalance in the plant water content could result in reduced growth and even in the total absence of growth under dry environmental conditions (Pamungkas *et al*., 2022). Tombesi *et al*. (2015) and Zargar *et al*. (2017), reported that decreasing water content causes loss of turgor pressure, cessation of cell enlargement, closure of stomata, limitation of carbon uptake, affects the photosynthetic rate, transpiration rate, stomatal conductance, and carboxylation efficiency, and interference with many other basic metabolic processes. Zhang *et al*. (2021) stated that drought stress inhibits chlorophyll synthesis and decreases chlorophyll content, having an impact on the role of photosynthetic apparatus. Unlike chlorophyll, xanthophyll is found to be less sensitive to drought stress conditions, and shown to be upregulated having a protective role and playing an inhibitory role on reactive oxygen species (ROS) production (Wang *et al*., 2018). Nikolaeva *et al*. (2010) reported that, during photosynthesis's light reaction process, the normality of pigments is important as they participate in the light energy absorption process, for further converting the solar energy into chemical energy to be stored in the form of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). The pigments commonly found in plants are chlorophyll-a, chlorophyll-b, and carotenoids. The activity of RuBisCO, the key enzyme for carbon metabolism in leaves, is reported to be strikingly decreased in conditions of water stress, subsequently reducing the fixation of CO_2 (Zhang *et al*., 2021). The activity of other photosynthetic enzymes like NAD-dependent malate dehydrogenase, phosphoenolpyruvate carboxylase, fructose-1,6-bisphosphatase (FBPase), and sucrose phosphate synthase (SPS) is found to be inhibited to different extents (Maxiselly *et al*., 2022). The change in starch and sucrose ratio causes alterations in the inorganic phosphorus (P*i*) flux across the chloroplast membrane. Thus, the reduction of P_i in the chloroplasts inhibits ATP synthesis with a great impact on the photophosphorylation and photosynthetic carbon reduction cycle (Chaves *et al*.,

2009). Drought stress also lowers the cyclic and noncyclic types of electron transport during the light reaction of photosynthesis, negatively affects the photophosphorylation process, and decreases ATP synthesis as well as $NADP^+$ reduction (Zargar *et al*., 2017). The cumulative effect of all these factors affects the intensity of photo-assimilation and the stability of the photosynthetic apparatus.

Under drought stress, in addition to the production of antioxidants, the osmotic adjustment occurs in plant cells through the accumulation of compatible solutes in the cytosol (Zhang *et al*., 2011). The term "compatible solutes" includes derivatives of sugars, amino acids, tri-carboxylic acid cycle, Glycolysis, γ-aminobutyric acid shut, Shikimic pathway, and others. All these compatible solutes are highly soluble and do not interfere with cell metabolism even at high concentrations (Guo *et al*., 2018). The content of soluble sugars and other carbohydrates in the leaves of various water-stressed plants is altered and may act as a metabolic signal in response to drought stress (Rabara *et al*., 2017). Studies have shown that soluble sugars accumulate in leaves during water stress and have suggested that these sugars might contribute to osmoregulation, at least under moderate stress (Fabregas and Fernie, 2019). Drought stress may inhibit the synthesis of different proteins equally while inducing the synthesis of a specific stress protein (Schwender, 2009). Dehydrins, Osmotin, and proline have been the most observed groups among the accumulated proteins in response to loss of water. Proline accumulation may represent a regulatory mechanism of water loss by reducing the cell water potential; however, it also serves as a biochemical marker of metabolic changes caused by stress (Szabados and Savoure, 2010). Zhang *et al*. (2017) concluded that drought stress inhibits amino acid utilization, and they are accumulated, giving a 10–100-fold accumulation of free asparagine, valine, and glutamic acid, but alanine levels decreased.

Along with proteins, lipids are the most abundant component of membranes, and they play a role in the resistance of plant cells to environmental stresses (Zhang *et al*., 2011). Investigations on various crop species record a general decrease in phospholipid, glycolipid, and linoleic acid contents and an increase in the triacylglycerol of leaf tissues exposed to long periods of water deficits (Araujo *et al*., 2012). The most comprehensive information about the mechanism of regulation of gene expression in response to water deficit has been obtained from the investigation of DNA elements and sequence-specific DNA-binding proteins (Min *et al*., 2019). Presently, two classes of DNA elements have been identified: the ABA-responsive element (ABRE) and the dehydration-responsive element (DRE) (Deokar and Taran, 2016). The ABRE is sufficient for ABA-regulated gene expression during water deficit, but in some genes, it must be associated with a coupling element (Rodrigues *et al*., 2013).

1.3. Statement of the Problem

Coffee genotypes are considered to be drought-sensitive, and they possess different affinity mechanisms to alleviate and survive drought stress conditions. Coffee genotypes have substantial variation in tolerance to drought stresses in terms of accumulation and production of heat shock proteins, antioxidant enzymes, osmolytes and osmotic adjustment, stomatal conductance, photosynthesis, carbohydrate metabolism, respiration, energy production, and yield (DaMatta *et al*., 2001; Moustakas *et al*., 2022).

Hence, developing and screening cultivars capable of withstanding drought stress and producing enough yields is therefore of utmost importance (Wrigley, 1995; DaMatta and Ramalho, 2006; WCR, 2021). According to WCR (2021), coffee breeding programs have developed and identified cultivar that withstands drought stress conditions. Physiological studies revealed that drought-tolerant cultivars are characterized by deep root systems, improved tissue water status,

associated with maintenance of leaf area, adequate stomatal control of water use, and improved long-term water-use efficiency (WUE) as soil water becomes limiting (DaMatta *et al*., 2003; Mirian *et al*., 2006; Fahad *et al*., 2017; Xiong *et al*., 2022).

In Ethiopia, most of the research on the effects of drought stress in coffee reported in the past has mainly focused on agronomical practices, water use, or the physiology of leaves, and consequences on yield, but findings related to the responses and characterization of coffee's (*C. arabica*) germination, growth, physiology, metabolite and gene expression towards drought stress were rarely conducted. Recently, studies in the the impact of drought stress on the physiology and metabolism of coffee, that ultimately determine yield, are rising. However, there is limited information on the effects of drought stress on the germination, physiology, and molecular characterization of the Ethiopian coffee genotypes. Besides, the knowledge of drought tolerance of Ethiopian coffee genotypes is crucial for maintaining production in coffee-producing regions of Ethiopia, where water supply is limited (Moat *et al*., 2017). Therefore, further studies are still needed for a better understanding of the Ethiopian *C. arabica* genotypes.

Hence, at the seed level, this study aims to investigate the morphological and developmental changes occurring during germination and post-germination periods of coffee genotypes that possess varying physiological responses to drought conditions. At seedling levels, the study also aims to investigate the expression of growth performances and physiology of arabica coffee genotypes towards drought stress. The study also intends to characterize the responses of metabolites under drought-stress conditions. Based on these characterizations, the study intends the elucidation relevant physiological and molecular mechanisms that enable the coffee genotypes to adjust to drought; and identify coffee genotypes that have the potential for not only withstanding the water deficit but also for establishing in drought-intensified coffee regions of Ethiopia.

1.4. Research Questions, Hypotheses and Objectives

1.4.1. Research questions

- How does the seed germination characterstics and subsequent performance of seedling vary among *C. arabica* genotypes?
- What constitutes the growth, physiological, and biochemical responses of *C. arabica* genotypes towards drought-stressed conditions?
- Do *C. arabica* genotypes have different biochemical and metabolite responses under under drought-stressed conditions?

1.4.2. Research objectives

1.4.2.1. General objective

Investigating and characterizing the responses of seed germination, growth, physiological, and biochemical responses *C. arabica* genotypes under induced drought-stress conditions.

1.4.2.2. Specific objectives

The specific objectives of the study are to:

- Evaluate and characterize the seed germination characterstics and subsequent performance of *C. arabica* seedling under induced drought-stress conditions;
- Evaluate and characterize the mechanisms underlying the growth, physiological and biochemical responses of relatively tolerant and sensitive *C. arabica* genotypes to induced drought-stress conditions; and,
- Evalute and characterize the composition, accumulation and alteration of metabolites in relatively tolerant and sensitive *C. arabica* genotypes under drought-stress conditions.

1.5. Outline of the Thesis

This dissertation is organized into five main chapters.

Chapter 1 - General Introduction

The origin, taxonomy and description, occurrence and distribution, and threats for the growth of *C. arabica* plants are described. The impacts of drought stress on seed germination, morpho-physiological, and molecular responses of *C. arabica* genotypes are presented. In addition, this part includes the research questions, objectives, and outline of the dissertation.

Chapter 2 - Differences in Germination Performances of Coffee (*Coffea arabica* L.) Genotypes with Different Tolerance Levels to Drought Stress

This chapter characterizes seed traits of *C. arabica* genotypes and investigates their responses to germination and post-germination events while under drought stress conditions. Seed traits that determine the germination efficiency, morphological transformations observed during germination stages, germination-indicating factors, and post-germination seedling performances are described and characterized.

Chapter 3 - Impact of Drought Stress on Growth and Physiology in Coffee (*Coffea arabica* L.) Genotypes with Varying Drought Tolerance

This chapter investigates the growth, biomass, water relations, gas exchanges, pigments, stomatal densities, and cell membrane stability responses of the *C. arabica* genotypes towards drought stress conditions. The genotype's drought tolerance and sensitivity indices are evaluated to develop a basis for screening tolerant genotypes.

Chapter 4 - Drought Stress Responses in Arabica Coffee (*Coffea arabica* L.) Genotypes: Physiological and Metabolic Insights

Drought-tolerant and sensitive *C. arabica* genotypes are characterized in terms of metabolic alterations in response to drought stress through GC–MS analysis techniques. Metabolic alterations are associated with other biometrics responses towards drought stress conditions.

Chapter 5 - General Discussion, Conclusion and Recommendation

This chapter compiles and discusses the main findings reported in chapters 2-4. Besides, it also contains recommendations and future research directions. Following this chapter, references and appendices are displayed.

2. CHAPTER TWO

Differences in Germination Performances of Coffee (*Coffea arabica* L.) Genotypes with Different Tolerance Levels to Drought Stress

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Abstract

Seed germination is a transitional process that awakens dormant seeds, allowing to resume metabolic activities and protrude their radicles. Studies have shown that the process of seed germination and subsequent seedling growth are highly sensitive to moisture availability. Moreover, some seeds including arabica coffee seeds, are known to have asynchronous and slow germination, which are their natural characteristics. This study investigated the germination and early vegetative growth performance of three different groups of coffee genotypes, i.e. relatively tolerant (*Ca*74140, *Ca*74112, and *Ca*74110), moderately sensitive (*Ca*74158, *Ca*74165, and *Ca*J-21), and sensitive (*Ca*754, *Ca*J-19, and *Ca*Geisha), behaving differently in their physiological response to a dry soil condition. The germination ratio assay was conducted in a pot experiment, using a randomized complete block design. Relatively tolerant genotypes exhibited quicker and more complete germination than the other groups. Germination percentage, which was higher in relatively tolerant genotypes compared to the sensitive groups, was strongly associated with seed traits such as moisture content (r = 0.965), the seed's surface area (r= 0.901), and the seed's surface area to volume ratio (r= 0.852). The tolerant genotypes exhibited higher coefficients of the velocity of germination, coefficient of variation of germination time, germination rate index, and germination index; however, they also had lower synchrony of the germination process compared to the sensitive genotypes. Furthermore, higher seedling vigor during the early stage of development in the relatively tolerant genotype groups was observed. Hence, the indicators of germination performance and early seedling growth can be utilized to screen genotypes that are tolerant to drought conditions.

Keywords: Arabica coffee, drought, genotype, germination, moisture content, seedling vigor, seeds

2.1. Introduction

Coffea arabica L. is the most widely-cultivated commercial species, accounting for over 70% of the world's coffee production (Esayas Aga *et al*., 2003; WCR, 2021). It is believed that the South and Southwest of Ethiopia is the center of origin and genetic diversity for *C. arabica* (Smith, 1985; Tesfaye Shimber, 2018[b]). Coffee plants thrive best in areas where an altitude ranges between 1,600-2,800 m, rainfall is high, humidity is between 50% and 80%, light intensity is moderate, and slightly acidic soil is present (Taye Kufa, 2012). Ethiopia is the leading Arabica coffee producer in Africa and the tenth-largest exporter worldwide, producing an average of ~ 471,000 tons per year with a yield of 0.71 tons/ha (ECX, 2021). Ethiopian coffee is highly sought-after for its superior quality and organic nature (Samuel Gebresselasei and Eva, 2008; CSA, 2021; ECX, 2021).

Although coffee production is strongly affected by drought events, a significant portion of the world's coffee has been cultivated in drought-prone regions where the use of irrigation is the exception (DaMatta *et al*., 2007). Countries like Ethiopia are facing serious irrigation problems due to the predicted increases in the frequency and severity of drought episodes and increased temperatures which in turn are expected to augment the air evaporative demand and thus affect soil water availability (Justin *et al*., 2017). Drought stress that negatively impacts coffee growth is the major constraint and a growing concern to bean production and yields (Mirian *et al*., 2006). Since coffee plants are sensitive to environmental changes, the germination process, seedling establishment as well as plant development are highly vulnerable (Rosa *et al*., 2010).

The propagation of *C. arabica* plants is usually done through their seeds (Karssen *et al*., 1989; Rosa *et al*., 2010). When the external and internal conditions necessary for seed germination are suitable, healthy, and properly stored seeds will germinate effectively (Giorgini

and Campos, 1992; Linkies *et al*., 2010). However, soil moisture has a significant impact on the coffee seed germination process and seedling emergence. The effect of soil moisture on the activation of the embryo and the subsequent radicle development may substantially differ among coffee genotypes (DaMatta *et al*., 2007; Etienne *et al*., 2013; Baskin and Baskin, 2014). During the process of imbibitions, hydrophilic molecules (-OH, -NH_2, -COOH, etc.) accumulate beneath the hard external layer of the coffee endosperm and draw in water molecules. This causes a build-up of turgor potential within the seed but further expansion is inhibited by an opposing mechanical force of the surrounding endosperm (Takaki and Dietrich, 1980; Silva *et al*., 2008; Steinbrecher and Leubner-Metzger, 2017).

However, the water molecules initiate the mobilization of endogenous gibberellic acids towards the soft internal endosperm region, leading to the synthesis of hydrolyzing enzymes (endo-β-mannanase, cellulase, amylase, and protease) to break the endosperm cell wall surrounding the embryo and create space for the embryo expansion and elongation, and weakening of the endosperm cap leading to the development of coffee seed protuberance (Takaki and Dietrich, 1980; Steinbrecher and Leubner-Metzger, 2017). Subsequently, the stored food reserves (carbohydrates, proteins, and lipids) break down into simpler biomolecules, such as simple sugars, amino acids, and fatty acids (Finch-Savage and Leubner-Metzger, 2006; Schopfer, 2006; Weitbrecht *et al*., 2011; Etienne *et al*., 2013). These simpler biomolecules then move toward the growing embryo, where they become metabolically active in the developing tissue (Mirian *et al*., 2006; Voegele *et al*., 2012).

Uniformity in seed germination and seedling vigor is essential for the successful establishment of commercial crops. However, coffee seeds are naturally characterized by asynchronous and slow germination (Silva *et al*., 2005). This slow germination is caused by the

loss of germination capacity and other related factors. In tropical rain-fed areas of arid and semiarid regions, soil moisture is the primary factor determining seed germination (DaMatta *et al*., 2007). Additionally, the efficiency of seed germination, among the coffee species and genotypes, is influenced by the permeability of the endosperm (hard external and soft internal layer), temperatures, air moisture, seed moisture, seed damage, and other factors (Rosa *et al*., 2010; Weitbrecht *et al*., 2011).

Germination is a key developmental process that determines all subsequent developmental stages. Therefore, understanding the morphological and developmental changes occurring during coffee seed germination, as well as the key germination-indicating factors associated with this process, is essential for the improvement of germination practices under drought-affected environments. This study aimed to examine the germination and post-germination periods of some selected coffee genotypes that possess varying physiological responses to drought conditions.

2.2. Materials and Methods

2.2.1. Study site

The study was conducted in the greenhouse of Plant Biology and Biodiversity Management at the College of Natural and Computational Sciences of Addis Ababa University, with a 40% shade level (an average temperature of 22.3°C and a photon flux density of 725 ± 11 μmol $m^{-2}s^{-1}$) that mimicked the natural conditions of major coffee-growing regions in Ethiopia (WCR, 2021). In the greenhouse, the germination experiment was conducted within a poly-propagator wooden box (5m x 1m x 1m, sand layered covered with polyethylene plastic sheet) which was maintained at a temperature of 26°C, humidity of 55%, and a photon flux density of 345 ± 16 μmol $m^{-2}s^{-1}$, with a 12-hour photoperiod.

2.2.2. Plant material

Nine *C. arabica* L. (*Ca*) genotypes obtained from the Jimma Agricultural Research Center (JARC) were used in this study. These genotypes were selected based on their drought tolerance: relatively tolerant (genotypes signed: *Ca*74140, *Ca*74112, and *Ca*74110), moderately sensitive (*Ca*74158, *Ca*74165, and *Ca*J-21), and sensitive (*Ca*754, *Ca*J-19, and *Ca*Geisha) (Tesfaye Shimber, 2018[b]) (Appendix 1). According to WCR (2021) and ISTA (2005), ripe and healthy berries from young, vigorous, and disease-free coffee plants were collected from the respective coffee genotype, the berry skin (pericarp/exocarp) properly removed, the pulp (mesocarp) and mucilage properly washed, without removing the parchment/hull (endocarp) and silver skin (spermoderm) the seed/bean (endosperm) were sealed in a plastic bag and stored under a refrigerator 1-4°C with a relative humidity of less than 40%, before the germination experiment. These processes kept the seed's initial moisture content from desiccation.

Pre-germination parameters such as seed length (Sl) and seed width (Sw) (using a digital caliper to the nearest 0.01 mm), and seed fresh (Fw) and seed dry weight (Dw) (using balance to an accuracy of 0.01 g) were measured. The seed's initial moisture content (Mc), surface area (SA), and volume (SV) were calculated using the following equations (ISTA, 2005) (Appendix 2):

Seed initial moisture content (%):

$$Mc = \left(\frac{F_w - D_w}{D_w}\right) \times 100 \quad (1)$$

Where F_w is the seed fresh weight and D_w is the seed dry weight. Mc was calculated based on the loss in weight as a percentage of the dry weight of the seeds.

Surface area (mm^2):

$$SA = Sl \times Sw \quad (2)$$

Where Sl is the seed length and Sw is the seed width.

Seed Volume (cm^3):

$$SV = \frac{\pi Sl Sw^2}{6} \quad (3)$$

Where Sl is the seed length and Sw is the seed width, assuming that the width is equal to thickness.

2.2.3. Germination performance

The sand was sieved with a 2 mm sieve, thoroughly washed with tap water, and sterilized in an oven at around 180°C for 3 hours. After cooling to room temperature, it was spread thinly on germination plastic trays (5 cm deep) to allow for radicle development (Legesse Negash, 2010; Etienne *et al*., 2013). Before sowing, parchment (endocarp), and silver skin (spermoderm) of the fresh seeds were removed, followed by the sterilization of the endosperm using a mixture of 95% ethanol and 30% hydrogen peroxide (1:1, v:v) for 10 minutes and cold-distilled-water imbibitions for 12 h (Michiel *et al*., 2004) (Appendix 3).

The germination experiment was started in August 2021. Twenty seeds of each genotype were sown on plastic trays at a depth of one cm in three replications. The trays were placed in a randomized complete block design within a poly-propagator. To prevent spatial effects, the trays were moved randomly within the poly-propagator once a week. The trays were irrigated daily until the germination phase reached the “matchstick” size (before the emergence of cotyledons). The germinants were then transplanted into growing pots containing composite soil (topsoil, compost, and sand in a ratio of 2:1:1; pH of 5.4-6.8) with a perforated (9 mm diameter holes) held under the pot to allow drainage. Daily trial management and observation of germination

were performed until the radical of each seed reached 2 mm in length, signifying the completion of the germination (WCR, 2021) (Appendix 4 and 17).

Germination parameters such as germination percentage (GP) (Scott *et al*., 1984), the mean germination time (MGT) (Ellis and Roberts, 1981), coefficient of variation of germination time (CV_t) (Ranal and Santana, 2006; Ranal *et al*., 2009), coefficient of the velocity of germination (CVG) (Jones and Sanders, 1987), germination index (GI) (Bench *et al*., 1991), germination rate index (GRI) (Esechie, 1994), the uncertainty of germination process (U) (Labouriau and Valadares, 1976; Kader, 1998), synchrony of germination process (Z) (Labouriau, 1978), mean daily germination percent (MDG) (Adams and Farrish, 1992), peak value for germination (Pv) (Adams and Farrish, 1992), and germination value (Gv) (Czabator, 1962), were calculated based on the following formulas (Appendix 5):

Germination percentage (%):

$$\mathrm{GP} = (\sum_{i=1}^{k} \mathrm{n}_i/\mathrm{N}) \times 100 \quad (4)$$

Where n_i is the number of seeds germinated in the i^{th} time, N is the number of all seeds that completed germination and k is the total number of time intervals.

Mean germination time (day):

$$\mathrm{MGT} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \quad (5)$$

Where t_i is the time from the start of the experiment to the i^{th} interval, n_i is the number of seeds germinated in the i^{th} time interval (not the accumulated number, but the number corresponding to the i^{th} interval), and k is the total number of time intervals.

Coefficient of variation of germination time (%):

$$\mathrm{CVt} = \frac{\mathrm{S_t}}{\mathrm{MGT}} \quad (6)$$

Where S_t is the standard deviation of germination time and MGT is the mean germination time.

Coefficient of velocity of germination (%):

$$\mathrm{CVG} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i} \times 100 \quad (7)$$

Germination index (day):

$$\mathrm{GI} = \sum_{i=1}^{k} n_i / t_i \quad (8)$$

Where n_i is the number of seeds germinated in the i^{th} time and t_i is the time needed for seeds to germinate at the i^{th} count.

Germination rate index (%/day):

$$\mathrm{GRI} = \frac{\mathrm{G1}}{1} + \frac{\mathrm{G2}}{2} + \cdots \frac{\mathrm{Gn}}{\mathrm{n}} \quad (9)$$

Where G_1 is the germination percentage at the first day after sowing and G_2 is the germination percentage × 100 at the second day after sowing, G_n is the germination percentage at the n day after sowing.

Uncertainty of germination process (degree of uncertainty) (bit):

$$\mathrm{U} = \sum_{i=1}^{k} f_i \log_2 f_i \quad (10)$$

Where $f_i = \frac{n_i}{\sum_{i=1}^{k} n_i}$, f_i is the relative frequency of germination, n_i is the number of seeds germinated in the i^{th} time interval, and k is the total number of time intervals.

Synchrony of germination process (degree of overlapping):

$$\mathrm{Z} = \frac{\sum_{i=1}^{k} C_{cni,2}}{\mathrm{C}_{\sum \mathrm{ni},2}} \quad (11)$$

Where $C_{ni,2} = n_i(n_i\text{-}1)/2$, $C_{ni,2}$ is the partial combination of the two germinated seeds from among *ni*, from the number of seeds germinated on the i^{th} time interval, $C_{\sum ni,2}$ is the partial combination

of the two germinated seeds from among the total number of seeds germinated at the final count, assuming that all seeds that germinated did so simultaneously.

Mean daily germination percent (%):

$$\mathrm{MDG} = \frac{\mathrm{GP}}{\mathrm{Tn}} \tag{12}$$

Where GP is the final germination percentage and T_n is the total number of intervals required for final germination.

Peak value (% time^{-1}):

$$\mathrm{Pv} = \max\left(\frac{G1}{t1}, \frac{G2}{t2}, \ldots \frac{Gk}{tk}\right) \tag{13}$$

Where t_i is the time from the start of the germination to the i^{th} interval, G_i is the cumulative germination percentage in the i^{th} time interval and k is the total number of time intervals.

Germination value (%^{2} time^{-1}):

$$\mathrm{Gv} = \mathrm{MDG} \times \mathrm{Pv} \tag{14}$$

Where MDG is the mean daily germination and Pv is the peak value.

2.2.4. Post-germination events

Post-germination parameters, at 90 days of growth, such as root length (RL), shoot length (SdL), the ratio of root/shoot length (R/S^r), number of lateral roots (RN) were also measured. The vigor index (VI) (Allan *et al*., 1962), were calculated based on the following formula:

Vigor index:

$$\mathrm{VI} = (\mathrm{SdL} + \mathrm{RL}) \times \mathrm{GP} \tag{15}$$

Where SdL is the mean shoot length, RL is the mean root length, and GP is germination percentage.

Seedling morphological changes during germination and post-germination phases were also photographed using SonyAlphaA7RIV (Sony Group Corporation, Thailand) and observed under a Leica MZ8 microscope (Leica Microsystems Ltd, Switzerland) at 100 dpi resolution.

2.2.5. Statistical analysis

Analysis of variance (ANOVA), Tukey's honest significant difference (HSD) at $p<0.05$ significance level, Pearson correlations and principal component analysis (PCA) analysis were used. All statistical analysis was performed using SigmaPlot version 13 (Systat Software Inc., San Jose, CA, US).

2.3. Results

2.3.1. Evaluating quality traits of *C. arabica* seeds across different genotypes

The analyses of germination potential-associated parameters (see Figure 2.1) showed significant differences ($p<0.05$) in the seed's dry weight (Dw), length (Sl), surface area (SA), volume (SV), moisture content (Mc), and SA to SV ratio between the tested coffee genotypes, except for fresh weight *(*Fw) and seed width (Sw). The highest values for Dw (see Figure 2.1A), Sl (see Figure 2.1B), SA (see Figure 2.1C), SV (see Figure 2.1D), and Mc (see Figure 2.1E) were recorded in the relatively tolerant genotype *Ca*74140 (0.174 g, 9.9 mm, 68.31 mm^2, 0.247 cm^3, and 14.89%, respectively), while the lowest values of those parameters were recorded in the sensitive genotype *Ca*754 (0.149 g, 7 mm, 51.8 mm^2, 0.201 cm^3, and 8.05%, respectively). Additionally, the highest SA to SV ratio (see Figure 2.1F) of seeds was recorded in genotype *Ca*74140 (0.277), and the lowest in genotype *Ca*754 (0.258).

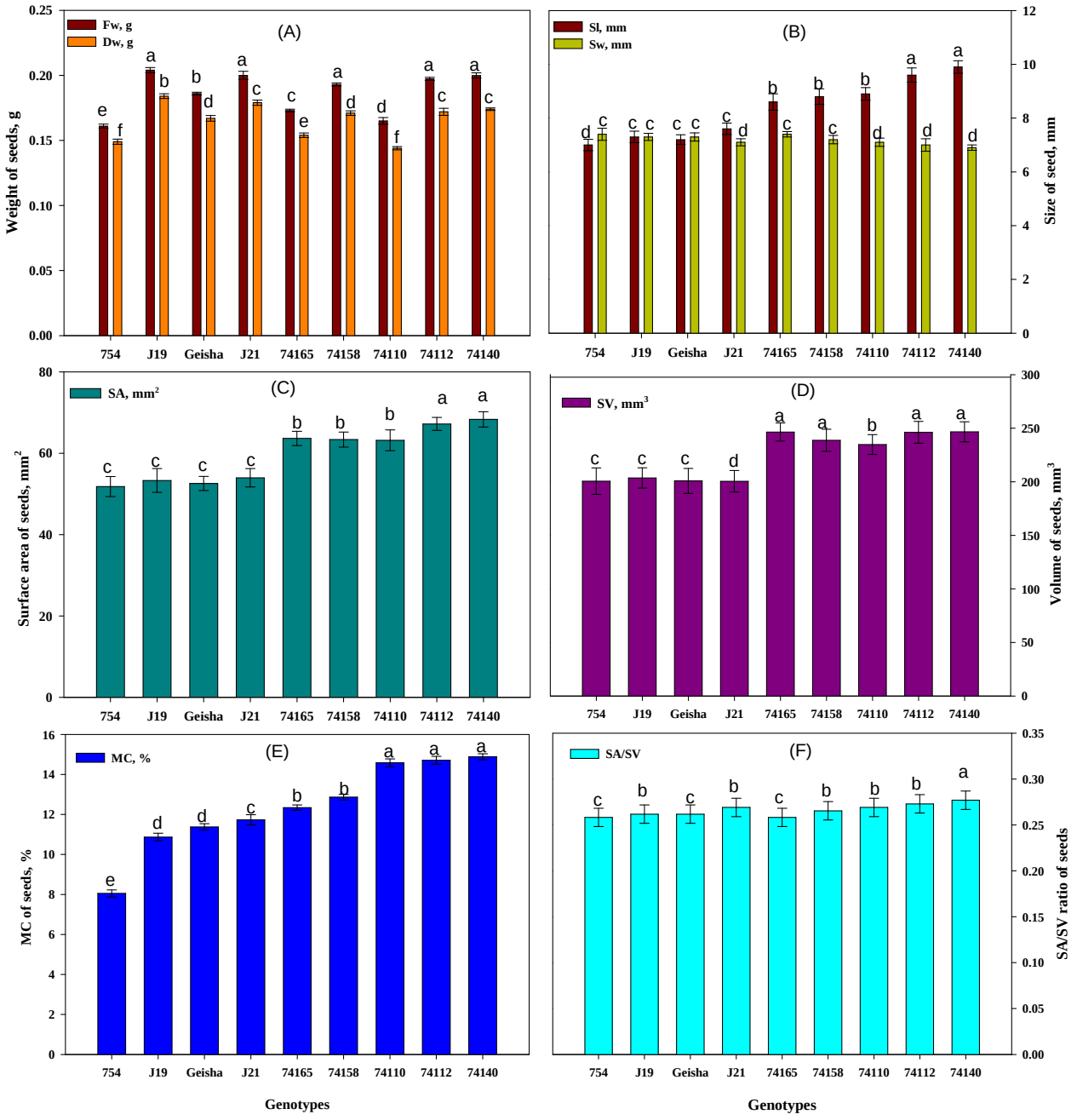

Figure 2.1. Pre-germination parameters of the seeds of nine *C. arabica* genotypes: (**A**) mean fresh (Fw) and dry (Dw) weight, (**B**) length (Sl) and width (Sw), (**C**) surface area (SA), (**D**) volume (SV), (**E**) moisture content (Mc), and (**F**) surface area to volume ratio (SA/SV). Bars indicate means±SD, and the mean data are measurements of 60 representatives. Bars with the same letter do not differ significantly at $p<0.05$.

2.3.2. Evaluating variations in germination and post-germination events of *C. arabica* genotypes

The comparison of the duration of germination and post-germination events of tested *C. arabica* genotypes has revealed significant differences. Genotype *Ca*74112 belonging to relatively tolerant genotypes had the shortest time to complete each germination stage i.e. before germination stage-1 (*bg*-1) 3.2 days, before germination stage-2 (*bg*-2) 5.13 days, germination stage (*g*) 9.5 days, seedling development stage-1 (*sd*-1) 12.6 days, seedling development stage-2 (*sd*-2) 15.49 days, seedling development stage-3 (*sd*-3) 17.3days, seedling development stage-4 (*sd*-4) 22.3 days, and seedling development stage-5 (*sd*-5) 44.26 days compared to the moderately sensitive and sensitive genotypes. The longest time to complete each germination stage was observed for the sensitive genotype *Ca*754 (*bg*-1 5.94 days, *bg*-2 11.1 days, *g* 17.52 days, *sd*-1 20 days, *sd*-2 23 days, *sd*-3 26 days, and *sd*-4 32 days), besides the last stage *sd*-5 which was the longest for *Ca*J19 (53.2 days). The other two relatively tolerant genotypes i.e. *Ca*74110 and *Ca*74140 also had shorter periods to complete each developmental stage compared to the sensitive genotypes (see Table 2.1).

Apart from the time needed for each coffee genotype to complete germination and post-germination stage, all the assessed genotypes displayed similar patterns of morphological changes (see Figures 2.2 and 2.3, showing relatively tolerant *Ca*74112 as model genotype). At the *bg*-1 stage, the seeds of all genotypes fully imbibe water molecules without the appearance of a visible protuberance (see Figure 2.2A). At the *bg*-2 stages, the visible protuberance with a bulged root apex was noticed inside the endosperm cap caused by the elongation and growth of the embryo (see Figure 2.2B). Next, due to the increased metabolic activities growth of the embryo is highly facilitated, and the radicle with distinct features of the

embryonic axis and remnants of suspensor emerges out of the endosperm, indicating the end of the germination phase (see Figure 2.2C, D).

At the *sd*-1 stage (see Figure 2.3A), the endosperm area opposite the endosperm cap is positioned towards gravity (positive geotropism) and begins to swell due to the growing cotyledonary leaves. Anthocyanine-driven pink-colored hypocotyl begins to grow from the white arrow-shaped radicle, lifting the whole endosperm upward from the germinating media indicating an epigeal type of germination. At the *sd*-2 stage (see Figure 2.3B), root primordia appeared at the junction between the hypocotyl and primary root, and the radicle and hypocotyls became enlarged. At the *sd*-3 stage (see Figure 2.3C), the furrow in the outer endosperm begins to split and crack due to the growing pressure of the cotyledonary leaves. The endosperm begins to erect, the hypocotyl begins to change color into green, lateral roots develop from the root primordial regions, and additional root primordia and root hairs develop from the primary root. At the *sd*-4 stage (see Figure 2.3D), the outer structural parts of the endosperm become soft, flaccid, and loose. The structure of the endosperm containing the cotyledonary leaves becomes erect (positive phototropism) and hypocotyl begins changing color into green. Moreover, properly developed primary and lateral roots are present, and several root hairs appear on the surface of the root. At the *sd*-5 stage (see Figure 2.3E), the endosperm begins to disappear, folded cotyledonary leaves begin to open, and an increased number and size of primary and lateral roots are observed.

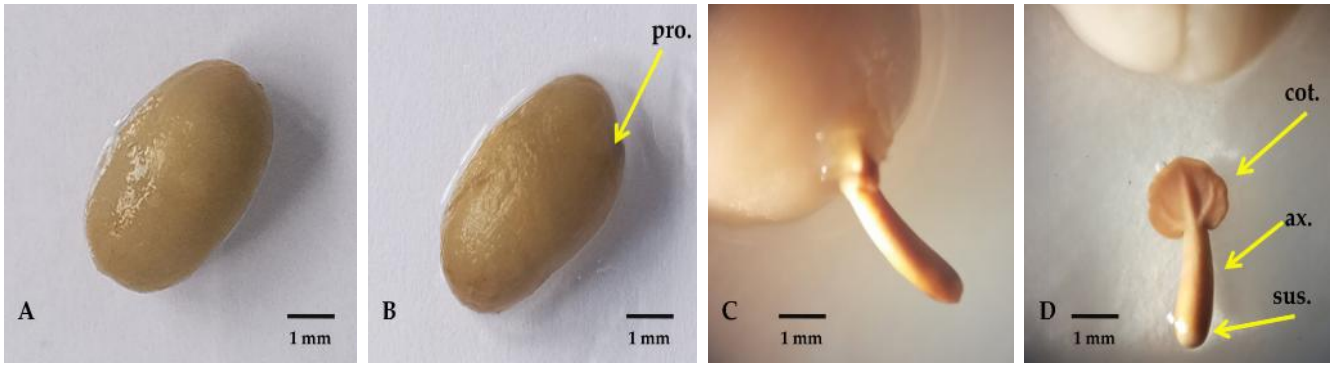

Figure 2.2. Photographs and micrographs of *C. arabica* germinating seed and embryo of relatively tolerant *Ca*74112 genotype: (**A**) seed during imbibition (*bg*-1), (**B**) imbibed seed with visible protuberance (pro.) (*bg*-2), (**C**) emergence of radicle from the outer layer of the endosperm (*g*), and (**D**) embryo with the cotyledons (cot.), the embryonic axis (ax.), and remnants of the suspensor (sus.) at the radicle tip (approximately 2-3.5 mm). Photos of A and B were taken using SonyAlphaA7RIV, and for C and D the observations were conducted under a Leica MZ8 microscope with a resolution power of 100 dpi.

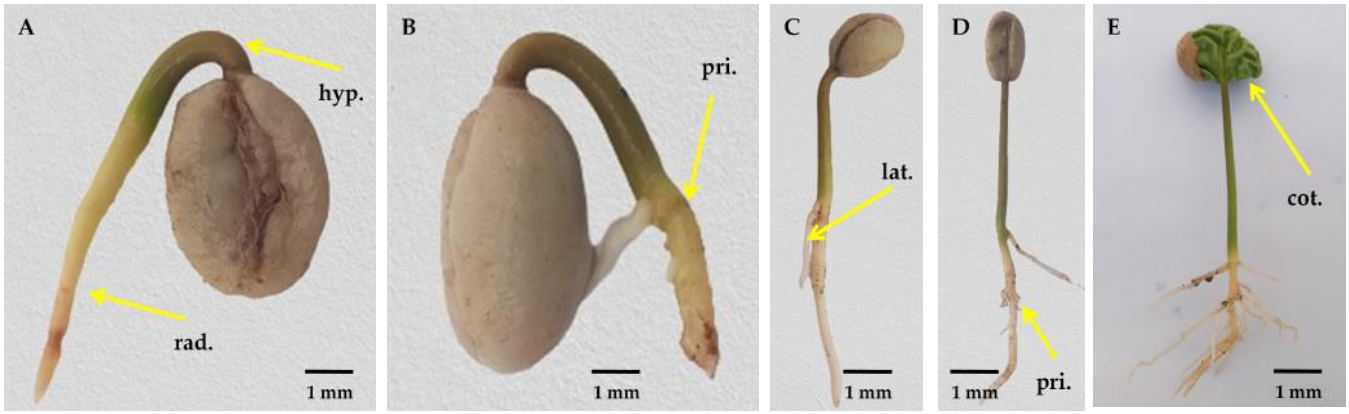

Figure 2.3. Photographs of post-germination stages of *C. arabica* development in chronological order of relatively tolerant *Ca*74112 genotype: (**A**) radicle (rad.) and hypocotyls (hyp.) emergence (*sd*-1), (**B**) root primordia (pri.) development between primary root and hypocotyls (*sd*-2), (**C**) lateral roots development (lat.) and appearance of root hairs on the primary root (*sd*-3), (**D**) properly developed primary (pri.) and lateral roots (*sd*-4), and (**E**) opening of cotyledonary (cot.) leaves (*sd*-5). Pictures were taken using SonyAlphaA7RIV.

Table 2.1. Chronological stages of before, during, and post-germination events of coffee seeds for nine *C. arabica* genotypes. The abbreviations *bg*, *g*, and *sd* - stand for physiological and morphological changes before germination, during germination, and during seedling development, respectively. Numbers represent means±SD for n=60 replicates per genotype. Numbers in each row with the same letter(s) are not significantly different at $p<0.05$.

Stage index	Stage name	Average period (days) of coffee seed developmental stages								
		*Ca*754	*Ca*J19	*Ca*Geisha	*Ca*J21	*Ca*74165	*Ca*74158	*Ca*74110	*Ca*74112	*Ca*74140
bg-1	Imbibition 1-primary imbibed seed	5.94±0.83^{b}	5.70±0.61^{b}	6.01±0.47^{c}	5.3±0.21^{b}	5.2±0.28^{b}	5.3±0.27^{b}	4.01±0.22^{a}	3.2±0.28^{a}	4.2±0.23^{a}
bg-2	Imbibition 2- visible protuberance	11.1±0.71^{d}	11.02±0.63^{d}	10.0±0.52^{c}	8.2±0.37^{c}	7.25±0.32^{b}	9.3±0.44^{c}	7.0±0.29^{b}	5.13±0.47^{a}	7.0±0.34^{b}
g	Germinated seed	17.52±0.27^{c}	16.27±0.21^{c}	16.09±0.36^{c}	13.78±0.32^{b}	13.33±0.25^{b}	14.15±0.35^{b}	10.07±0.26^{a}	9.5±0.24^{a}	11.61±0.24^{a}
sd-1	Seedling 1-arrow shaped	20±2.16^{c}	19.3±2.86^{c}	19.1±2.51^{c}	16±1.98^{b}	16±1.64^{b}	17.2±1.87^{b}	13.5±1.92^{a}	12.6±1.98^{a}	14.1±1.61^{a}
sd-2	Seedling 2-root primordia emergence	23.0±2.92^{c}	22.0±1.78^{c}	22.0±2.82^{c}	19.11±2.31^{b}	19.13±1.35^{b}	20.2±2.04^{b}	16.53±2.42^{a}	15.49±1.26^{a}	17.36±1.24^{a}
sd-3	Seedling 3-lateral roots emergence	26.0±2.31^{d}	25.0±1.98^{d}	25.26±1.78^{d}	22.7±2.34^{c}	22.2±2.74^{c}	23.1±1.56^{c}	19.2±1.36^{b}	17.3±1.21^{a}	20.14±2.04^{b}
sd-4	Seedling 4-lateral roots development	32.0±2.09^{d}	31.1±2.11^{d}	30.21±2.05^{d}	28.2±1.28^{c}	27.05±1.37^{c}	28.1±2.27^{c}	24.6±2.42^{b}	22.3±2.39^{a}	25.0±2.75^{b}
sd-5	Seedling 5-photosynthetic cotyledons appear	51.2±3.07^{c}	53.2±3.86^{c}	50.02±3.54^{c}	47.31±3.22^{b}	49.27±2.84^{b}	49.3±3.05^{b}	46.0±3.01^{a}	44.26±3.21^{a}	46.0±2.23^{a}

2.3.3. Assessing seed germination potential indicators of the different genotypes

Significant variations in the germination parameters were recorded among the nine *C. arabica* genotypes analyzed in this study. In general, relatively tolerant genotypes *Ca*74140, *Ca*74112, and *Ca*74110 demonstrated higher germination performances compared to moderately sensitive (*Ca*74158, *Ca*74165, and *Ca*J-21), and sensitive (*Ca*754, *Ca*J-19, and *Ca*Geisha) genotypes (see Figure 2.4).

Germination percentage (GP) is an estimate of the germinability of the population of seeds (Scott *et al*., 1984), and the GP values calculated for tested coffee genotypes were as follows: relatively tolerant i.e. *Ca*74140 (90±1.44%), *Ca*74112 (80±1.74%), *Ca*74110 (75±1.56%), moderately sensitive i.e. *Ca*74158 (65±1.85%), *Ca*74165 (60±1.75%), *Ca*J-21 (55±2.92%), and sensitive i.e. *Ca*Geisha (50±2.46%), *Ca*J-19 (45±2.21%), and *Ca*754 (35±2.67%) (see Figure 2.4A). Mean germination time (MGT) which is a measure of the time it takes for the seed to germinate, focusing on the day at which most seeds have germinated (Ellis and Roberts, 1981) was the shortest for the relatively tolerant genotype *Ca*74112 (9.50 days) and significantly longer for sensitive genotype *Ca*754 (17.52 days) (see Figure 2.4B). CVG focuses on the time required to reach the final germination percentage (Jones and Sanders, 1987), and CV_t interprets and calculates the coefficient of variation of the mean germination time (Ranal *et al*., 2009). Hence, in terms of CVG and CV_t, the highest values were recorded in the relatively tolerant genotype *Ca*74112 (10.53% and 20.94%, respectively) and the lowest values were observed insensitive genotype *Ca*754 (5.71% and 2.92%, respectively) (see Figure 2.4C). GRI describes the percentage of germination per day, the higher the percentage and the shorter the duration leads to higher GRI (Esechie, 1994), whereas germination index (GI) is an estimate of the time (in days) it takes a certain germination percentage to occur (Bench *et al*., 1991). In this study, the highest

and lowest values of GRI and GI were recorded in the relatively tolerant genotype *Ca*74112 (8.75%/day and 5.25 seed/day, respectively) and the lowest in the sensitive genotype*Ca*754 (1.99%/day and 1.2 seed/day, respectively) (see Figure 2.4D).

Uncertainty of germination (U), indicates the degree of uncertainty associated with the distribution of relative frequency of germination (Labouriau and Valadares, 1976), and synchrony of germination process (Z) describes the degree of overlapping of germination among seeds (Labouriau, 1978). The highest U values were recorded in the relatively tolerant genotype *Ca*74110/*Ca*74112 (2.61 bit), whereas the lowest value was observed for moderately sensitive genotype *Ca*J-21 (0.76 bit), respectively (see Figure 2.4E). The highest Z value was recorded in the moderately sensitive genotype *Ca*J-21 (0.64) and the lowest for relatively tolerant genotype *Ca*74112 (0.19), respectively (see Figure 2.4E). The mean daily germination (MDG) percent represents the mean number of seeds germinated per day (Adams and Farrish, 1992). The MDG of the relatively tolerant genotype *Ca*74140 exhibited the highest (3.60%) and sensitive genotype *Ca*754 showed the lowest value (1.40%) (see Figure 2.4F). The peak value (Pv) of germination is the accumulated number of seeds germinated at the point on the germination curve at which the rate of germination starts to decrease (Adams and Farrish, 1992), and germination value (Gv) is the combination of speed and completeness of germination (Czabator, 1962). The relatively tolerant genotype *Ca*74140 exhibited the highest Pv and Gv (6.54% day^{-1} and 23.54%^{2} day^{-1}, respectively) and sensitive genotype *Ca*754 showed the lowest Pv and Gv values (1.94% day^{-1} and 2.72%^{2} day^{-1}, respectively) (see Figure 2.4G).

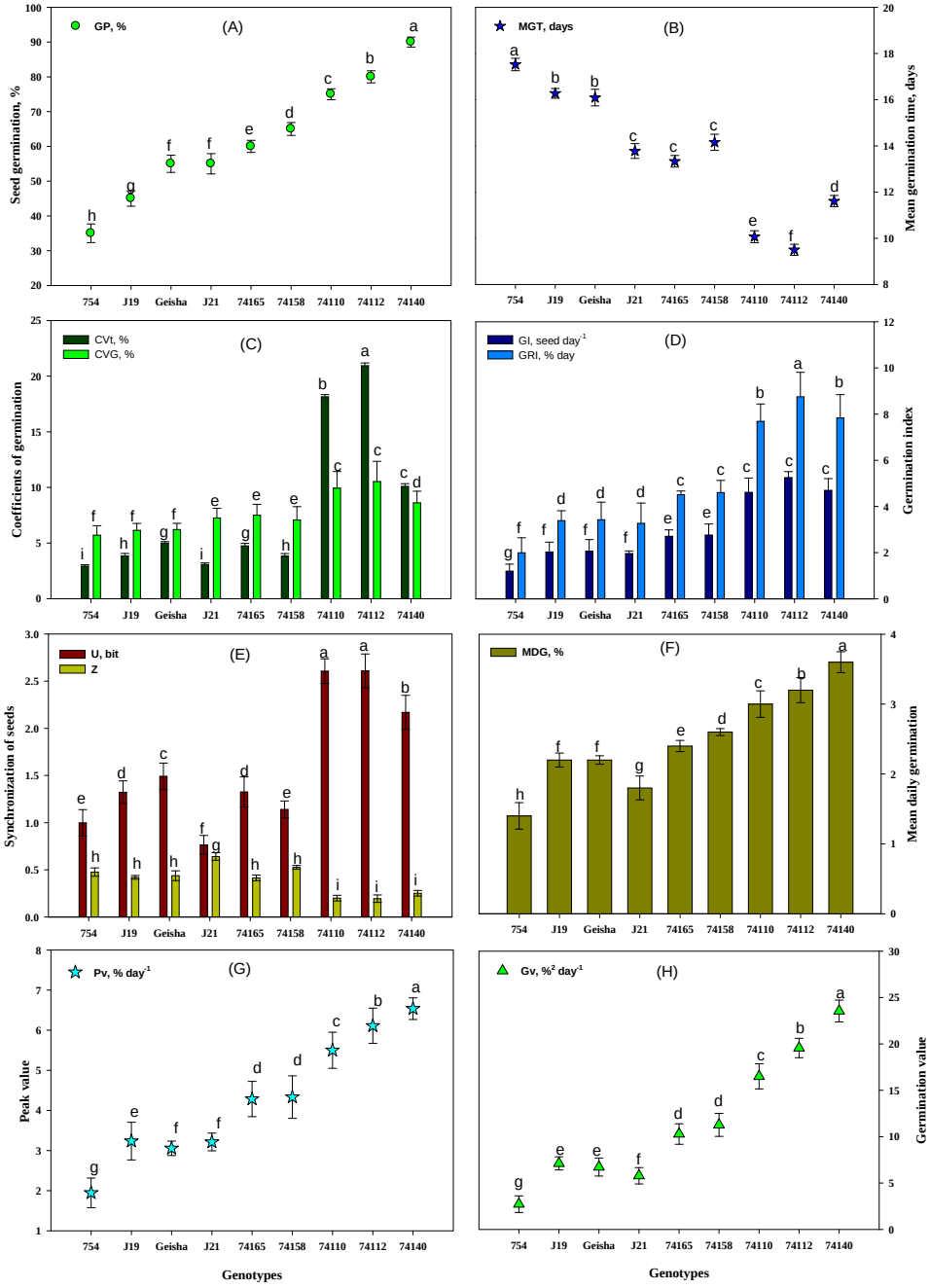

Figure 2.4. Germination parameters of the nine *C. arabica* genotypes: (**A**) mean germination percentage (GP), (**B**) mean germination time (MGT), (**C**) coefficient of variation of germination time (CV_t) and coefficient of the velocity of germination (CVG), (**D**) germination index (GI)- and germination rate index (GRI), (**E**) uncertainty of germination process (U) and synchronization index (Z), (**F**) mean daily germination percent (MDG), (**G**) peak value for germination (Pv), and (**H**) germination value (Gv). Dots and bars indicate means±SD (n=60 replicates per genotype). Dots and bars with the same letter do not differ significantly at p<0.05.

2.3.4. Assessing seedling performances

Coffee seedlings were collected after 90 days of seed sowing. The result revealed significant differences (p<0.05) in seedlings of different genotypes as shown by root and shoot length (RL and SdL), root number (RN), and vigorous index (VI). The highest RL, SdL, and RN were recorded in the relatively tolerant genotypes *Ca*74112 (67.5 mm, 50 mm, and 22.5, respectively), *Ca*74110 (65.75 mm, 49.75 mm, and 22.25, respectively), and *Ca*74140 (62.75 mm, 48 mm, and 22.75, respectively), while the lowest was observed in sensitive genotype *Ca*754 (45 mm, 42.75 mm, and 13.5, respectively). Additionally, the highest root-to-shoot ratios (R/S^r) were calculated in relatively tolerant genotypes *Ca*74112 (1.35), *Ca*74110 (1.32), and *Ca*74140 (1.31), and the lowest was found in sensitive genotype *Ca*754 (1.05). Moreover, relatively tolerant genotype *Ca*74140 had the highest VI (9967.5), followed by *Ca*74112 (9400) and *Ca*74110 (8662.5), while the significantly lower VI was recorded for t he sensitive genotype *Ca*754 (3071.25) (see Table 2.2).

Table 2.2. Root (RL, mm) length, shoot (SdL, mm) length, root number (RN), root-shoot ratio (R/S^r), and vigorous index (VI) of 90-day-old seedlings representing nine coffee genotypes. The numbers are means±SD of 60 representatives of each genotype. Data in the same column with the same superscript are not significantly different at p<0.05.

Genotypes	RL(mm)	SdL(mm)	RN	R/S^r	VI
***Ca*754**	45 ± 0.41^c	42.75 ± 0.1^b	13.5 ± 1.19^c	1.05	3071.25^d
***Ca*J19**	51.75 ± 0.21^b	42.75 ± 0.25^c	14 ± 2.46^b	1.21	4252.5^c
***Ca*Geisha**	52.5 ± 0.51^c	43.5 ± 0.23^b	15 ± 1.47^c	1.21	5280^c
***Ca*J21**	59 ± 0.21^c	46.5 ± 0.25^b	19.25 ± 2.46^c	1.27	5802.5^c
***Ca*74165**	60.75 ± 0.41^b	47.75 ± 0.23^c	21.75 ± 2.75^b	1.27	6510^b
***Ca*74158**	53.75 ± 0.53^c	44.25 ± 0.21^b	16 ± 1.58^b	1.21	6370^b
***Ca*74110**	65.75 ± 0.31^a	49.75 ± 0.09^a	22.25 ± 2.66^a	1.32	8662.5^a
***Ca*74112**	67.5 ± 0.30^a	50 ± 0.21^a	22.5 ± 3.08^a	1.35	9400^a
***Ca*74140**	62.75 ± 0.90^a	48 ± 0.20^a	22.75 ± 1.75^a	1.31	9967.5^a

2.3.5. Multivariate analysis

2.3.5.1. PCA and cluster analysis

The PCA analysis was performed on a data set containing measurements of seed quality traits, germination parameters, and 90-day-old seedlings measurements. The first principal component (PC1) accounted for 77.8% of the variation, separating relatively tolerant genotypes (*Ca*74140, *Ca*74112, and *Ca*74110) from the moderately sensitive and sensitive genotypes (see Figure 2.5). Among the tested traits, seed volume (SV), mean germination time (MGT), germination percentage (GP), coefficient of variation of germination time (CV_t), coefficient of the velocity of germination (CVG), germination rate index (GRI), and germination index (GI) were the largest contributors to the separation along PC1, while fresh (Fw) and dry (Dw) weight of seed had the highest contribution for the separation of genotypes along PC2 (see Figure 2.6).

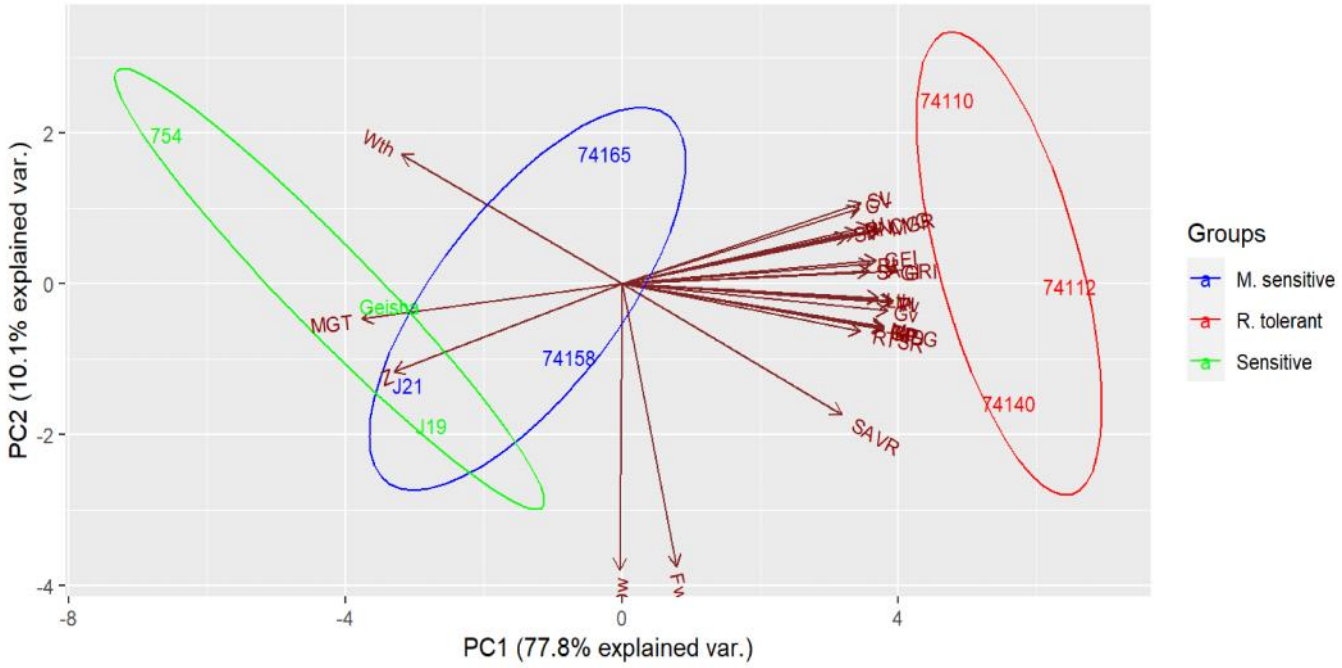

Figure 2.5. PCA biplot with the first two principal components of the nine coffee genotypes showing the clustering of genotypes and variables contributing to the separation. The percentage of the variance captured by each PC is given next to each respective axis.

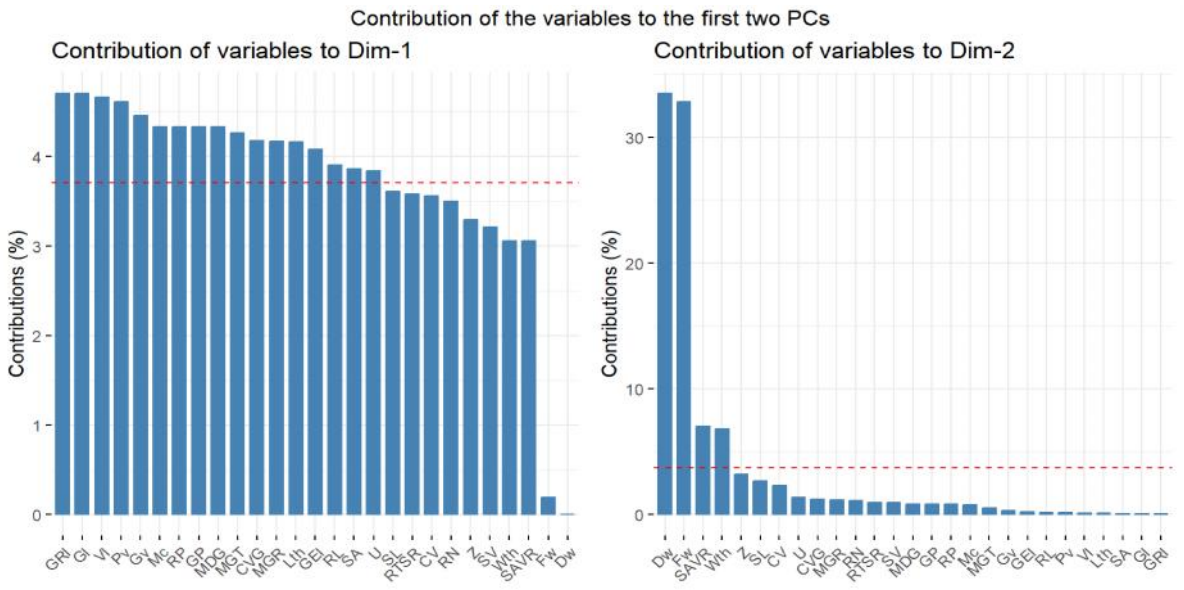

Figure 2.6. Contribution of pre-, during-, and post- germination variables for the two principal components.

2.3.5.2. Pearson correlation

The mean time of various coffee developmental stages, i.e., *bg*-1 (*r*= 0.959), *bg*-2 (*r*= 0.952), *sd*-1 (*r*= 0.991), *sd*-2 (*r*= 0.995), *sd*-3 (*r*= 0.991), *sd*-4 (*r*= 0.988), and *sd*-5 (*r*= 0.914), was strongly correlated with the mean germination time (MGT). Mc was positively and strongly correlated with Sl (*r*= 0.90), SA (*r*= 0.87), and SV (*r*= 0.79), and negatively correlated with MGT (*r*= -0.92), Sw (*r*= -0.80), and Z (*r*= -0.66).

GP was positively and strongly correlated with pre-germination parameters i.e. Mc (*r*= 0.97), SA (*r*= 0.90), Sl (*r*= 0.95), and SV (*r*= 0.81); germination parameters i.e. Pv (*r*= 0.98), Gv (*r*= 0.97), and MDG (*r*= 0.96); and post-germination parameters i.e. RL (*r*= 0.86) and R/S^r (*r*= 0.84). However, GP was weakly correlated with Fw (*r*= 0.282), and negatively correlated with MGT (*r*= -0.89), Sw(*r*= -0.85), and Z (*r*= -0.68). VI was also positively correlated with Mc (*r*= 0.81) and GP (*r*= 0.99), and negatively correlated with MGT (*r*= -0.94), Sw (*r*= -0.84), and Z (*r*= -0.73) (see Figure 2.7).

r	Dw	Mc	Sl	Sw	SA	SV	SA/SV	GP	MGT	CVt	CVG	GI	U	Z	MDG	Pv	Gv	GRI	RL	SdL	RN	R/Sr	VI
Fw	0.98	0.31	0.20	-0.50	0.12	0.01	0.50	0.28	-0.11	-0.05	0.05	0.16	-0.04	0.12	0.30	0.26	0.25	0.16	0.18	-0.03	0.04	0.38	0.23
Dw		0.10	0.00	-0.34	-0.07	-0.17	0.33	0.07	0.10	-0.22	-0.14	-0.05	-0.21	0.28	0.10	0.05	0.05	-0.05	-0.02	-0.22	-0.15	0.19	0.02
Mc			0.90	-0.80	0.87	0.79	0.80	0.97	-0.92	0.74	0.87	0.93	0.78	-0.66	0.94	0.96	0.92	0.93	0.91	0.83	0.84	0.93	0.97
Sl				-0.75	0.99	0.94	0.75	0.95	-0.87	0.68	0.83	0.92	0.72	-0.67	0.93	0.97	0.95	0.92	0.81	0.80	0.84	0.77	0.94
Sw					-0.64	-0.47	-1.00	-0.85	0.76	-0.63	-0.74	-0.79	-0.62	0.49	-0.77	-0.81	-0.82	-0.79	-0.72	-0.66	-0.66	-0.72	-0.84
SA						0.98	0.64	0.90	-0.84	0.65	0.80	0.88	0.69	-0.66	0.89	0.94	0.91	0.88	0.79	0.79	0.82	0.73	0.90
SV							0.47	0.81	-0.77	0.57	0.72	0.80	0.62	-0.62	0.82	0.86	0.83	0.80	0.72	0.73	0.77	0.66	0.81
SA/SV								0.85	-0.76	0.63	0.74	0.80	0.62	-0.49	0.77	0.81	0.83	0.80	0.72	0.66	0.67	0.72	0.85
GP									-0.89	0.71	0.84	0.93	0.77	-0.68	0.96	0.98	0.97	0.93	0.86	0.81	0.84	0.84	0.99
MGT										-0.87	-0.99	-0.94	-0.82	0.71	-0.82	-0.91	-0.86	-0.94	-0.97	-0.96	-0.92	-0.90	-0.94
CVt											0.94	0.90	0.95	-0.88	0.72	0.77	0.76	0.90	0.80	0.82	0.69	0.70	0.78
CVG												0.95	0.87	-0.77	0.79	0.88	0.84	0.95	0.94	0.95	0.87	0.85	0.91
GI													0.92	-0.86	0.94	0.97	0.96	1.00	0.89	0.86	0.83	0.83	0.96
U														-0.97	0.83	0.83	0.84	0.92	0.75	0.75	0.67	0.69	0.82
Z															-0.79	-0.77	-0.80	-0.86	-0.64	-0.66	-0.61	-0.56	-0.73
MDG																0.98	0.98	0.94	0.78	0.71	0.74	0.80	0.94
Pv																	0.99	0.97	0.87	0.83	0.85	0.85	0.98
Gv																		0.96	0.81	0.77	0.80	0.78	0.96
GRI																			0.89	0.86	0.83	0.83	0.96
RL																				0.97	0.95	0.96	0.91
SdL																					0.97	0.86	0.88
RN																						0.87	0.90
R/Sr																							0.88

Figure 2.7. Pearson correlation coefficients (***r***) of pre-, during- and post-germination parameters of the nine *C. arabica* genotypes of *Ca*74140, *Ca*74112, *Ca*74110, *Ca*74158, *Ca*74165, *Ca*J-21, *Ca*754, *Ca*J-19, and *Ca*Geisha.

2.4. Discussion

2.4.1. Seed trait variation associated with germination potential

The size and weight of the coffee seeds are usually influenced by both internal (i.e. genetic makeup, hormones, water content) and external factors (i.e. available water, storage techniques, etc). Both seed size and weight are correlated with the initial seed moisture content, surface area, volume, and germination percentage (DaMatta *et al*., 2007; Mirian *et al*., 2006). One of the key seed traits associated with germination is the moisture content, which is vital for determining the germination potential of coffee seeds (Finch-Savage and Leubner-Metzger, 2006). Seeds with higher moisture content provide efficient germination capacity and improve the seed's potential to tolerate drought conditions (Steinbrecher and Leubner-Metzger, 2017). In the current study, seeds of relatively tolerant genotypes retain significantly higher moisture content (i.e. *Ca*74140 14.89%, *Ca*74112 14.71%, and *Ca*74110 14.58%) than the other genotypes. Sensitive genotypes (*Ca*754 8.05%, *Ca*J-19 10.87%, and *Ca*Geisha 11.38%), on the other hand, retained the lowest seed moisture content which may be associated with their sensitivity to drought. A coffee seed with moisture content lower than 9% is characterized by having poor germination performance and high sensitivity to drought. Seeds with moisture content between 9-13% exhibit an improved germination potential but are still moderately sensitive to drought. When the moisture content is above 13%, the seeds will have much more reliable germination potential and are known to be drought-tolerant (ISTA, 2005; Steinbrecher and Leubner-Metzger, 2017).

Relatively drought-tolerant genotypes (*Ca*74140, *Ca*74112, and *Ca*74110) tend to have more elongated seeds than the sensitive genotypes in our study. Elongated seeds usually have a higher surface area-to-volume ratio. Previous studies had found a positive relationship between seed length and germination parameters (Wang *et al*., 2021). Seeds with a higher surface area-to-

volume ratio (i.e., relatively tolerant genotypes in this study) could absorb more water from the soil and germinate more quickly than those with a lower surface area-to-volume ratio (Harper *et al*., 1970). Elongated seeds with a high surface area-to-volume ratio will have more contact with the soil moisture and air which increases the potential of seeds to imbibe more water molecules so that they will have high germination potential. Meanwhile, genotypes with relatively heavier seed weight (e.g., *Ca*74140 and *Ca*74112) supposedly have more reserve foods (cellulose, hemicellulose, and insoluble mannan) inside the endosperm and potentially are more tolerant to environmental stress conditions (Silva, 2002; Mirian *et al*., 2006; Rosa *et al*., 2011). Similar to the previous findings (Linkies and Leubner-Metzger, 2012), our study affirms that genotypes having elongated seeds with higher surface area to volume ratio and higher mass like the relatively tolerant genotypes *Ca*74140, *Ca*74112, and *Ca*74110 are characterized by efficient germination even under drought stress conditions.

2.4.2. The speed of morphological changes is highly associated with genotypes

Slow and asynchronous germination of coffee seeds is in part due to the differences in the imbibitions of seeds during the germination process (Mirian *et al*., 2006; Schopfer, 2006). Hydrophilic molecules found in the outer and harder part of the coffee endosperm seed coat facilitate water absorption and cause the seed to become turgid and rounded (Mirian *et al*., 2006; Rosa *et al*., 2010) (see Figure 2.8). Furthermore, the endosperm wall stretches, resulting in structural changes in seeds in all dimensions. Some research also noted that coffee seeds with higher content of hydrophilic molecules in the endosperm get hydrated and germinate faster even under limited water availability or in drought stress conditions (DaMatta *et al*., 2007). In the present study, the relatively tolerant genotypes (i.e., *Ca*74112, *Ca*74110, and *Ca*74140) had the shortest hydration time, and are likely to contain more hydrophilic compounds, enabling them to

germinate quickly and withstand water shortages. Studies have suggested that faster hydration of coffee a seed allows more oxygen to enter the embryo and activates aerobic respiration (Silva *et al*., 2008; Weitbrecht *et al*., 2011; Steinbrecher and Leubner-Metzger, 2017). This process triggers the activation of hydrolyzing enzymes that catalyze the hydrolysis of food reserves in the endosperm (Takaki and Dietrich, 1980; Currey, 2005; Steinbrecher and Leubner-Metzger, 2017), resulting in the transformation of the quiescent embryo to a metabolically active one (Bewley and Black, 1994; Barry-Etienne *et al*., 2002; Silva, 2002; Silva *et al*., 2008). Other experiments also suggested that the difference in the rate of imbibition and hydration has a direct effect on the germination speed and the growth rate of coffee seedlings (Ronchi *et al*., 2006; Baskin and Baskin, 2014).

The initial germination events such as water imbibitions, O_2 entry, sub-cellular structural changes, molecular synthesis, and cellular respiration lead to cell division (Mirian *et al*., 2006; Etienne *et al*., 2013; Steinbrecher and Leubner-Metzger, 2017), and radicle development that breaks through the outer layer of the endosperm (Linkies and Leubner-Metzger, 2012; Voegele *et al*., 2012). The fastest germination observed in the relatively tolerant genotypes (*Ca*74112, *Ca*74110, and *Ca*74140) could be attributed to a shorter period of initial germination events. The higher value of mean germination time is correlated with rapid seed germination even under drought stress conditions and with high tolerance capacity during the early germination period (Bewley and Black, 1994; DaMatta *et al*., 2007). Consequently, relatively tolerant genotypes completed the subsequent post-germination events (*sd*-1 to *sd*-5) earlier than the other genotypes. The early completion of germination events in these genotypes may allow the seedlings to grow rapidly and withstand a resource-limited environment (Rosa *et al*., 2010; Voegele *et al*., 2012).

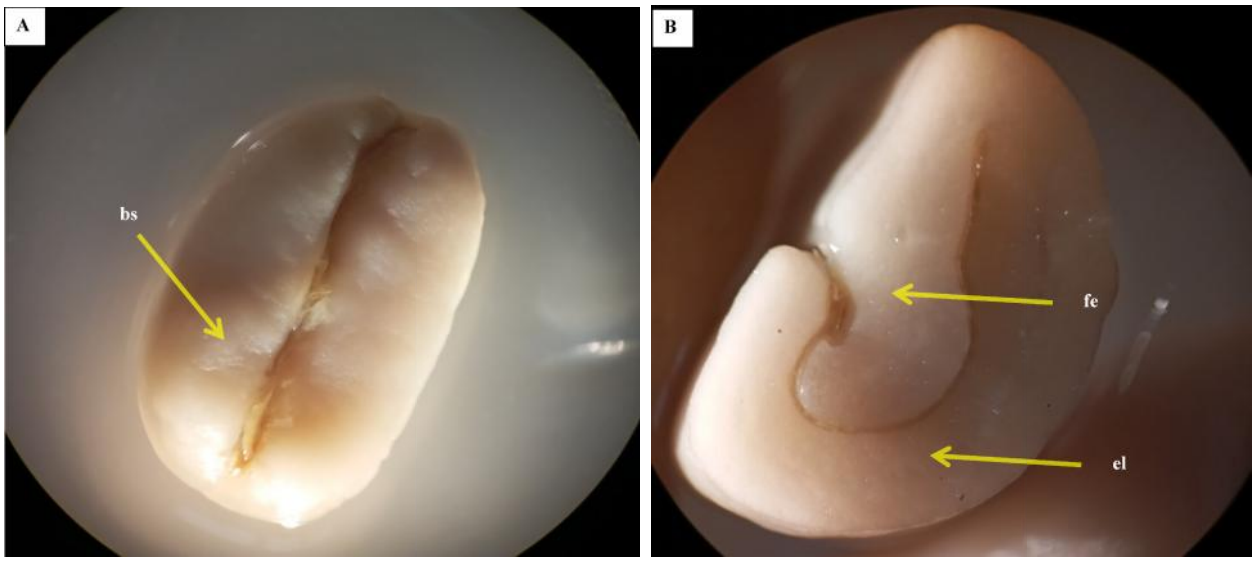

Figure 2.8. Microphotographs of: (**A**) basal surface (bs) of coffee seed endosperm, without exocarp, mesocarp, and endocarp, and (**B**) cross-section of a *C. arabica* seed showing the folding of the endosperm (fe) and embryo localization(el) during imbibitions process. Observations were conducted under a Leica MZ8 microscope with a resolution power of 100 dpi.

2.4.3. Germination performance variability is highly related to genotype

Germination percentage is a widely used parameter to predict the potential for germination and seedling establishment of a given lot of seeds (Huxley, 1964; Barry-Etienne *et al*., 2002; Hong and Ellis, 2002). The higher the germination percentage value the greater the germination of a seed population (Bewley and Black, 1994; Silva, 2002; Kader, 2005). Seeds with large surface area and volume are characterized by having an improved cellular division, elongation, differentiation, and growth that leads the seeds to attain high germination percentage (Bewley and Black, 1994; Mirian *et al*., 2006; DaMatta *et al*., 2007). In the current study, the germination percentage was higher in relatively tolerant genotypes and strongly correlated with the seed's surface area (r= 0.960), volume (r= 0.954), seed length (r= 0.918), and dry weight (r= 0.0728). For example, genotype *Ca*74140, with the highest moisture content, surface area, and volume exhibited the highest germination percentage (90±1.44%). Such genotypes are characterized by

the potential to produce seedlings that can tolerate stress conditions (Silva *et al*., 2004; Steinbrecher and Leubner-Metzger, 2017).

The mean germination time indicates the average duration of time required for the utmost seed germination performance i.e. lower values of mean germination time indicate faster germination (Giorgini and Campos, 1992; Etienne *et al*., 2013). This is directly related to the seed volume i.e., the higher volume of seeds the lower the mean germination time (Kader, 2005). For example, in this study, relatively drought-tolerant genotypes (*Ca*74140, *Ca*74110, and *Ca*74112) possess both higher moisture content and significantly lower mean germination time compared with the sensitive genotypes. The high volume of endosperm promotes faster germination i.e. lower mean germination time (Linkies *et al*., 2010). This is because the presence of a large food reserve allows for efficient metabolic activities, thus shortening the germination period and granting the capacity to grow even under drought-stress conditions (Silva *et al*., 2008; Steinbrecher and Leubner-Metzger, 2017). The PCA plot also showed that the relatively tolerant genotypes (*Ca*74140, *Ca*74112, and *Ca*74110) are separated from the sensitive genotypes (*Ca*754, *Ca*J-19, and *Ca*Geisha) along PC1 due to seed volume, mean germination time, germination percentage, coefficient of variation of germination time, coefficient of the velocity of germination, germination rate index, and germination index, presumably suggesting tolerance to drought stress. In this study, the coefficient of the velocity of germination and coefficient of variation of germination time was markedly higher in the relative drought-tolerant genotypes (*Ca*74112, *Ca*74140, and *Ca*74110) compared to the sensitive (*Ca*754, *Ca*J-19, and *Ca*Geisha) genotypes, indicating that germination was rapid but spread out over time in the tolerant genotypes. Additionally, genotypes *Ca*74112, *Ca*74140, and *Ca*74110 exhibited higher

germination rate index and germination index values, which are characteristics of drought-tolerant behavior (Kader, 2005; Etienne *et al*., 2013).

The mean number of seeds germinated per day (MDG) is directly associated and strongly correlated (r= 0.96) with the final germination percentage. The peak value (Pv) of germination (the maximum cumulative germination percentage per the number of total days) and mean daily germination can be counterbalanced, resulting in equal values for germination value (Gv) for samples or treatments with different behavior concerning the germination process (Brown and Mayer, 1988). Genotypes with higher Gv, like *Ca*74112, *Ca*74110, and *Ca*74140, have a high mean germination time and rapid vegetative growth, which can increase their capacity to withstand drought stress conditions (Ranal and Santana, 2006). The intrinsic traits of seed size and weight had a strong effect on mean germination time and germination percentage, which corroborates the relatively tolerant genotypes (*Ca*74140, *Ca*7412, and *Ca*74110). Moreover, the relatively tolerant genotypes display more vigorous growth and development of roots (high root branching root length, root number, accompanied by dense root hairs, etc) and shoots. In addition, a root-to-shoot ratio greater than 1 in relatively tolerant coffee genotypes (i.e., longer root than hypocotyls) is an indication of stable root-to-shoot balance. DaMatta *et al*. (2007) found that, a coffee seedling with such a shoot and root structure results in the plant developing higher shoot and root surface area and biomass. Well-developed roots provide efficient mechanical anchorage in the soil, improve the rate of nutrient and water uptake, boost the photosynthetic activity, and promote seedlings' plasticity to adapt to various environmental conditions, including drought stress (Tesfaye Shimber, 2018[b]; Giorgini and Comoli, 1996). In addition, some authors have considered, the development of such quality of root and shoot at early stages of development, like in the genotypes *Ca*74140, *Ca*74112, and *Ca*74110, have a far-

reaching influence on withstanding drought stress and thus improvingthe coffee yield (Giorgini and Campos, 1992; Mirian *et al*., 2006; Steinbrecher and Leubner-Metzger, 2017).

2.5. Conclusions

The present study revealed that the coffee plants belonging to different genotypes had notable differences in the period needed to finish their germination and post-germination stages of growth. In contrast, there were no important morphological differences between groups during the germination and post-germination stages. The relatively tolerant genotypes completed each stage of germination and post-germination seedling development faster and thus generated more robust seedlings compared to the moderately sensitive and sensitive groups. This can improve the plant's plasticity and ability to adapt to changing environmental conditions. The inherent qualities of the seeds are critical for their germination capability and post-germination development, yet the analysis of seed traits could be used to maximize the germination of coffee seeds and improve the yield of this economically important crop. Based on these findings, further research is recommended to investigate seed priming and microbial inoculations as potential methods to boost the slow germination process in *C. arabica*.

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3. CHAPTER THREE

Impact of Drought Stress on Growth and Physiology in Coffee (*Coffea arabica* L.) Genotypes with Varying Drought Tolerance

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Abstract

To investigate the growth and physiological responses to drought conditions, the study selected and used two relatively tolerant (*Ca*74110 and *Ca*74112) and sensitive (*Ca*J-19 and *Ca*754) *Coffea arabica* genotypes based on previous experiments where tolerance and sensitivity were assessed on soil drying at the early stages of coffee growth. The genotypes were subjected to growth under well-watered/*ww* and drought-stressed/*ws* conditions, and the impact on key growth, physiological, and biochemical performances were investigated. The result showed that, under drought-stress conditions; coffee growth, water relations, gas exchanges, chlorophyll contents, stomatal densities, and cell membrane stability responses of relatively tolerant and sensitive genotypes were significantly (p<0.05) lower than those growing under well-watered conditions. Comparative response among the genotypes under drought-stress condition showed that, as the intensity of drought intensifies, the relatively tolerant genotypes showed significantly (p<0.05) higher morpho-physiological and biochemical responses, compared to the sensitive genotypes (*Ca*J-19 and *Ca*754). Under drought stress conditions, stem length (r= 0.949), leaf number (r= 0.960), leaf area (r= 0.905), root length (r= 0.892), root number (r= 0.964), root volume (r= 0.827), net photosynthetic assimilation (r= 0.974), stomatal conductance (r= 0.944), transpiration rate (r= 0.973), chlorophyll-a (r= 0.950), chlorophyll-b (r= 0.903), stomatal densities (r= 0.931) and cell membrane stability (r= 0.981) were positively correlated with the Ψ_w, and were significantly (p<0.05) higher in the relatively tolerant genotypes, than the sensitive genotypes. The results indicated the growth, physiological, and biochemical performances of the coffee genotypes were significantly affected by drought stress conditions. Considering the main findings of the study, tolerance to drought stress should be the key factor in selecting, cultivating and scaling-up coffee genotypes, in the coffee producing agro-ecological zones, where drought stress is highly intensified.

Key words: drought stress, relative water content, biomass allocation, assimilation, stomatal density, cell membrane stability

3.1. Introduction

The optimal level of water availability is necessary for plants growth and development, and if the soil moisture content reaches the level (Ψ, <-0.5 MPa and below) causing drought stress, then it hampers the growth and development of the plant (Rodrigues *et al*., 2021). The effects vary with the duration and severity of the drought stress, growth stage and genotype, and their interactions (Nijabat *et al*., 2020).

Although coffee production is strongly affected by drought events, a significant portion of the world's coffee has been cultivated in drought-prone regions where the use of irrigation is the exception (DaMatta *et al*., 2007). Countries like Ethiopia are facing major irrigation problems due to drought intensification which in turn is expected to enhance the evaporative process and minimize the soil water availability (Justin *et al*., 2017).

The effects of drought stress include decreased leaf water content, turgor pressure, and cell division, elongation, and differentiation, as well as causing stomatal closure, reduced root-absorption capacity, reduced photosynthetic activity, and impaired metabolism, disrupt cell membrane stability, and electrolyte leakage that reduces cell viability leading to growth inhibition and mortality (Nijabat *et al*., 2020; da Silva *et al*., 2022).

Coffee plants, being one of the most sensitive plants towards drought stress, drought stress decreases the growth of stems, nodes, branches, leaf area, leaf size, leaf expansion, and causing leaf wilting and abscission leading to stunted growth, yield reduction, and death (da Silva *et al*., 2022). According to Seleiman *et al*. (2021) reported that root growth (number, elongation, and volume) are also influenced by drought stress. Hence, Mirian *et al*. (2006) reported greater taproot length in relatively tolerant coffee genotypes under drought stress conditions. A reduction of fresh and dry biomass is reported under drought conditions (DaMatta and Ramalho, 2006; Taiz and

Zeiger, 2010). Moreover, drought stress inhibits the development of floral structures, and the abortion of fruit (Moat *et al*., 2017).

Under drought stress, the root-to-shoot signaling facilitated by Abscisic Acid (ABA) hormones lowers stomatal conductance, which then reduces the rate of transpiration, lowers CO_2 internal concentrations, and decreases CO_2 fixation, so that the reduction of photosynthesis rate occurs (da Silva *et al*., 2022). Besides, the reduction of photosynthesis, under drought stress conditions, is associated with the reduction in the synthesis of adenosine triphosphate (ATP) and ribulose bisphosphate (RUBP) (Rodrigues *et al*., 2021; Xiong *et al.*, 2022). The reduction of photosynthesis rate is also associated with the reduction of chlorophyll synthesis and content, under drought stress conditions (Maxiselly *et al*., 2022). According to Xu and Zhou (2008) and Bertolino *et al*. (2019), stomatal size and densities are highly affected by drought stress, when leaf tissues undergo severe drought stress conditions, the number and the size of stomata reduce.

Moreover, the selection of genotypes capable of withstanding drought stress with sufficient growth and development is vital. However, selections of tolerant coffee genotypes have been largely empirical due to the limited discoveries of how coffee genotypes respond morphologically and physiologically to drought stress (DaMatta and Ramalho, 2006; Mirian *et al*., 2006). Despite the sufficient information on agronomic and yield characteristics, many questions about the performances of the Ethiopian arabica coffee genotypes towards drought stress conditions have been less thoroughly studied, and information relating to drought tolerance and response is scarce.

Therefore, the objectives of this study were to investigate the changes and responses in growth performances, water relations, gas exchange, photosynthetic pigments, stomatal densities, and cell membrane stability among the arabica coffee genotypes caused by drought stress, and identify

differences in morphological and physiological responses to drought tolerance and sensitivity among the coffee genotypes varying in their tolerance to drought stress.

3.2. Material and methods

3.2.1. Study site

The study was conducted in the green-house, mimicking the natural conditions of major coffee-growing regions in Ethiopia (WCR, 2021). The growth and physiological studies of adult coffee genotypes were conducted in a greenhouse with a mean temperature of 24.5°C, humidity of 50-70%, and photon flux density of 850 ± 13 µmol $m^{-2}s^{-1}$, with 12h light/12 h dark photoperiod.

3.2.2. Plant material

To examine the growth and physiological response of coffee genotypes during the adult stage, a follow-up experiment was conducted on selected relatively tolerant (*Ca*74110 and *Ca*74112) and sensitive (*Ca*754 and *Ca*J-19) groups. After germination, when the first pair of leaves appeared (with no traces of disease, hypocotyls (stem height of 3-5 cm), and roots with secondary roots (2-3 cm), seedlings were transplanted into 5 L plastic pots with an aluminum foil covering at the side and top to prevent excessive heat build-up and evaporative loss. Each pot was filled with a 4 L potting mix of topsoil, compost, and sand in a ratio of 2:1:1 and contained a perforated bottom (9 mm diameter holes) for drainage. Coffee seedlings were then managed in a greenhouse, as reported by WCR (2021) until the end of the experiment (Appendix 6, 18 and 19).

3.2.3. Experiment design

After developing 7-8 pairs of leaves (around 150 days of age), each genotype was subjected to two different conditions: a well-watered (*ww*) and drought-stressed (*ws*) condition. In the well-watered condition, the plants were irrigated to field capacity every 3-4 days and served as the control group. In the drought-stressed condition, the seedlings were initially fully irrigated at field water capacity

before the experiments began and then deprived of water until the end of the experiment. The experimental design was a completely randomized block design, forming a 4*2 factorial (four genotypes and two water applications), with a replication of 15 genotypes, comprising a total of 120 coffee plants. The pots were randomized regularly, and the interspacing between each coffee pot within each block was 0.5 m.

3.2.4. Growth performances

To evaluate the growth performance in response to droughtstress, at 10 days intervals till the end of the experiment (for around 60 days), measurements such as stem height (SH) and stem collar diameter (SD, at the collar of the plants) were measured using a meter scale and caliper (500-197-30, Mitutoyo group, Kanagawa, Japan), respectively, while leaf number (LN) were counted manually. The leaf area (LA) was calculated as proposed by Tavares-Junior *et al*. (2002).

$$LA = cE \quad (1)$$

Where E is an estimated area (E= length × width), and *c* is the coefficient index (*c*=0.99927).

At the end of the experiment (plants at around 210 days of age), the biomass of seedlings was assessed according to Taye Kufa (2012). The plants from the two treatments were uprooted between 9:00-11:00 AM and the roots were carefully excavated and cleaned with tap water over a 0.5 mm screen sieve. The fresh weights (root fresh mass-RFM, stem fresh mass-SFM, leaf fresh mass-LFM, and total fresh mass-TFM) were measured on a weighing balance (Sartorius, Germany), the tap root length (RL, line intersect method), root number (RN), and root volume (RV, using the water-displacement method in a graduated cylinder) were measured, counted and calculated.

$$RV = V_{war} - V_{wbr} \quad (2)$$

Where RV is the root volume, V*war* is the water volume after submerging the coffee roots into the graduated cylinder, and V*wbr* is the volume of the water in the graduated cylinder before submerging the coffee roots.

Further, the oven-dry mass (70°C for 24 h) of the root (RDM), stem (SDM), leaf (LDM), and total dry mass (TDM) of the coffee genotypes were measured. Based on the works of Poorter *et al*. (2011), dry matter content (DMC= dry mass/fresh mass, %), leaf mass fraction (LMF= leaf dry mass/total plant dry mass, g g^{-1}), stems mass fraction (SMF= stem dry mass/total plant dry mass, g g^{-1}), root mass fraction (RMF= root dry mass/total plant dry mass, g g^{-1}), root to shoot ratio (R/S ratio= root dry mass/(stem+leaf dry mass), g g^{-1}), specific stem length (SSL= stem length/stem dry mass, cm g^{-1}), specific root length (SRL= root length/root dry mass, cm g^{-1}), leaf area ratio (LAR= leaf area/total plant dry mass, cm^2 g^{-1}), specific leaf area (SLA= leaf area/leaf dry mass, cm^2 g^{-1}), and relative growth rate (RGR= increase in mass/unit mass/time, mg g^{-1} d^{-1}) were calculated to assess the impact of water stress on coffee's growth and development.

3.2.5. Leaf water potential

The stem water potential (Ψ_w) was measured at 9:00-11:00 AM of the day using a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, US), at 10 days intervals till the end of the experiment (for around 60 days). Due to the small size of the leaf petioles, only the stem water potential of each genotype was measured. The stems were excised using a sharp blade and placed into the pressure chamber. The chamber was pressurized using a nitrogen tank, and Ψ_w was recorded when the initial xylem sap was emerging from the cut end of the stem.

3.2.6. Leaf relative water content

Based on the works of Barrs and Weatherley (1962), at 10 day intervals till the end of the experiment (for around 60 days), relative water content (RWC) from representative leavesof the coffee genotypes was determined following the parameters:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100 \quad (3)$$

Where FW is the leaf fresh weight, DW is the leaf dry weight, and TW is the leaf turgid (re-saturated) weight.

The fresh weight of the leaves of the genotypes was measured, and for the determination of turgid weight, samples were soaked in distilled water for about 2 hours at room temperature (20-22°C) and weighed. Furthermore, for the determination of dry weight, the samples were dried to a constant weight at 70°C.

3.2.7. Gas exchange measurements

Instantaneous gas exchange measurements were periodically measured out at 10 days intervals till the end of the experiment (for around 60 days).The rate of net carbon assimilation (A_{net}, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs, mol H_2O m^{-2} s^{-1}), and transpiration rate (E, mmol H_2O m^{-2} s^{-1}), were collected using an open gas exchange system LI-6400 (LI-COR, USA) adjusted at 1000 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density, 400 µmol CO_2 mol^{-1} air reference CO_2 concentration, and 500 µmol s^{-1} flow rates.The measurements were conducted between 9:00-11:00 AM of the day, on a young and fully expanded leaf. Water use efficiency (WUE) was calculated from the A/E ratio (DaMatta *et al*., 2007).

3.2.8. Photosynthetic pigments

Following the protocols of Lichtenthaler (1987), for the analysis of pigment (chlorophylls), healthy and fully expanded leaf discs from the same leaves used for gas exchange measurements were collected and analyzed from the genotypes, at 0, 30, and 60 days after the start of drought treatment. Then photosynthetic pigments were extracted using 100% pure acetone and filtered using filter paper and using a double beam, the optical density was measured using a UV-Vis spectrophotometer (Model 3092, India) at 661.6 nm, 644.8nm, and 470 nm. The contents of chlorophyll a (Chl-*a*), chlorophyll b (Chl-*b*), and total chlorophyll were computed following the calculation:

$$Chla = 12.25A_{663.2} - 2.79A_{646.8} \quad (4)$$

$$Chlb = 121.5A_{646.8} - 5.10A_{663.2} \quad (5)$$

$$Tchl = \text{Chla} + \text{Chlb} \quad (6)$$

Where *Chl-a* is the content of chlorophyll-a (mg g^{-1} tissue*), Chl-b* is the content of chlorophyll-b (mg g^{-1} tissue), and *Tchl* is total chlorophyll content (mg g^{-1} tissue).

3.2.9. Stomatal densities

To determine leaf stomatal density, following the protocol of Radoglou and Jarvis (1990) and Taye Kufa and Burkhardt (2011), healthy mature leaves which were selected for gas exchange measurements were used, and impression approach was used. Using a cotton ball, the abaxial epidermal surface of the leaf was cleaned, gently smeared with nail polish in areas between the leaf's central vein and edge, and allowed to dry for about 5 min. The dried thin film was removed using transparent adhesive tape, mounted on a microscopic slide, and covered with a cover slip. Then to form an impression of the leaf surface, lightly pressure the slide with fine-point tweezers, and place it on an object slide and observe under an Olympus microscope (light microscope) at 400 × magnifications, with an eyepiece 10× and objective lens of 40×.

The field of view of diameter was measured by stage micrometer by the given objective, and the area was calculated. Then the numbers of stomata were counted, and stomatal density (the number of stomata per unit area, (mm^{-2})) were recorded within a small field of view (0.15 mm^2).

$$\mathrm{StD} = \mathrm{N.St.}/\mathrm{A} \quad (7)$$

Where StD refer to stomatal density, A is an area of the microscope field of view (A = Πr^2, where Π (pi) is a constant 3.14 and r is the radius of the microscope field of view), and N.St. refers to the number of stomata within the field of view.

3.2.10. Cell membrane stability

Based on the works of Nijabat *et al*. (2020), cell membrane stability (CMS) of young leaves was determined at the end of drought stress experiments (plants at around 210 days of age) through relative conductivity. Fully expanded leaves were cut into 1 cm^2 pieces, washed with tap water and distilled water, then placed in a vial containing 10ml de-ionized water for 18 h at 10°C.Then leaf discswere placed in vials at 25°C, and in a water bath at 50°C for 1 h 15 min, respectively. Then the leaveswere incubated at 15°C for 18 h to facilitate the diffusion of electrolytes from leaf tissue to aqueous media. Then the vials were brought to room temperature and initial conductance (ws_1 and ww_1) was measured after a brief shaking of the vials. Samples were then autoclaved at 0.10 MPa at 120°C for 10 min, cooled down to 20°C, contents were shaken, and final conductance (ws_2 and ww_2) was measured using a conductivity meter (HORIBA, model B-173).

Cell membrane stability (CMS, %) and relative cell injury (RCI, %) were calculated with the formulas:

$$\mathrm{CMS} = \left(\frac{1-\frac{ws1}{ws2}}{1-\frac{ww1}{ww2}}\right) \times 100 \quad (8)$$

$$RCI = 100 - CMS \quad (9)$$

Where *ws* and *ww* refer to conductance values for drought-stressed and well-watered coffee plants, respectively; and the numbers 1 and 2 refer to the initial and final conductance measurements, respectively.

3.2.11. Statistical analysis

Analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) at $p<0.05$ significance level were used. All statistical analyses were performed using SigmaPlot version 13 (Systat Software Inc., San Jose, CA, US). Pearson correlations and principal component analysis (PCA) were performed by Past 4.03 (Hammer *et al*., 2001).

3.3. Results

3.3.1. Shoot growth of coffee plants in control and drought stress conditions

Compared with the well-water plants, significant reductions were recorded in stem length, stem diameter, leaf number, and leaf area among the genotypes growing under drought-stress conditions. Under drought stress conditions, the highest stem length, stem diameter, leaf number, and leaf area (see Table 3.1) were recorded in the relatively tolerant genotype *Ca*74112 (18.14±0.04 cm, 3.34±0.08 cm, 9.5±0.5, 18.49±0.38 cm^2), and the lowest values of stem length, leaf number, and leaf area were recorded for sensitive genotypes *Ca*754 (11.38±0.3 cm, 8, 10.72±0.18 cm^2) and steam diameter for *Ca*J-19 (2.84±0.08 cm). Drought stress-induced minimum and maximum stem elongation and leaf area expansion were recorded in the genotype *Ca*754 (49.16%, 53.99%) and *Ca*74112 (61.67%, 68.81%), respectively (Appendix 7, 8 and 20).

Table 3.1. The mean stem height, stem diameter, leaf number, and leaf area of plants belonging to the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well-water (*ww*) and drought stress conditions (*ws*). The numbers are means±SD (n=15 replicates per genotype). Data in the same row with the same superscript are not significantly different at $p<0.05$. DADB indicates the number of days after drought stress begins.

Variable	Time (DADB)	*Ca*754		*Ca*J-19		*Ca*74110		*Ca*74112	
		ww	*ws*	*ww*	*ws*	*ww*	*ws*	*ww*	*ws*
Stem height (cm)	**0**	3±0.20^{a}	3±0.24^{a}	4.9±0.09^{b}	4.88±0.21^{b}	7.1±0.2^{c}	7±0.13^{c}	9.28±0.08^{d}	9.75±0.05^{d}
	10	4.25±0.13^{a}	4.2±0.31^{a}	6.13±0.05^{b}	6.12±0.2^{b}	8.3±0.21^{c}	8.22±0.11^{c}	10.49±0.08^{d}	10.96±0.06^{d}
	20	7.68±0.14^{a}	7.57±0.28^{a}	9.53±0.06^{b}	9.45±0.23^{b}	11.7±0.2^{c}	11.54±0.08^{c}	13.85±0.09^{d}	14.32±0.04^{d}
	30	11.61±0.15^{a}	8.59±0.28^{e}	13.48±0.05^{b}	10.45±0.2^{a}	15.65±0.18^{c}	12.55±0.11^{b}	17.8±0.06^{d}	15.31±0.04^{c}
	40	15.7±0.15^{a}	9.88±0.26^{c}	17.58±0.07^{a}	11.75±0.21^{c}	19.76±0.2^{b}	13.85±0.11^{d}	21.92±0.08^{b}	16.6±0.03^{e}
	50	18.08±0.17^{a}	10.91±0.29^{c}	19.99±0.07^{a}	12.76±0.21^{c}	22.15±0.2^{b}	14.86±0.12^{d}	24.3±0.06^{b}	17.6±0.04^{e}
	60	23.15±0.20^{a}	11.38±0.3^{c}	25.08±0.09^{a}	13.25±0.19^{c}	27.21±0.18^{b}	15.35±0.09^{d}	29.41±0.07^{b}	18.14±0.04^{e}
Stem diameter (mm)	**0**	1.96±0.05^{a}	1.9±0.11^{a}	1.57±0.06^{a}	1.61±0.08^{a}	2.05±0.09^{a}	2.04±0.21^{a}	2.23±0.16^{a}	2.08±0.05^{a}
	10	2.43±0.03^{a}	2.41±0.13^{a}	1.98±0.09^{a}	2.03±0.12^{a}	2.58±0.08^{b}	2.52±0.23^{b}	2.71±0.19^{b}	2.56±0.05^{b}
	20	2.9±0.06^{a}	2.8±0.11^{a}	2.48±0.06^{b}	2.53±0.09^{b}	3±0.09^{c}	2.97±0.2^{c}	3.18±0.2^{c}	2.9±0.09^{c}
	30	3.44±0.05^{c}	3.03±0.12^{a}	3.0±0.07^{a}	2.74±0.07^{a}	3.51±0.05^{d}	3.17±0.22^{a}	3.69±0.19^{e}	3.26±0.07^{b}
	40	3.88±0.05^{a}	3.07±0.11^{c}	3.47±0.06^{a}	2.76±0.08^{c}	3.98±0.09^{b}	3.20±0.22^{d}	4.16±0.2^{b}	3.29±0.07^{d}
	50	4.39±0.03^{a}	3.12±0.11^{c}	3.98±0.07^{a}	2.82±0.09^{c}	4.49±0.05^{b}	3.24±0.22^{d}	4.69±0.18^{b}	3.31±0.07^{d}
	60	4.88±0.05^{a}	3.11±0.12^{b}	4.49±0.06^{a}	2.84±0.08^{b}	5.02±0.05^{a}	3.27±0.21^{c}	5.19±0.18^{a}	3.34±0.08^{d}
Leaf number	**0**	6±0.0^{a}	6±0.0^{a}	6.5±0.5^{a}	6.5±0.4^{a}	7±0.51^{b}	7±0.54^{b}	7.5±0.5^{c}	7.5±0.3^{c}
	10	8±0.0^{a}	8±0.0^{a}	8.5±0.4^{b}	8.5±0.5^{b}	9±0.53^{c}	9±0.58^{c}	9.5±0.5^{d}	9.5±0.3^{d}
	20	8±0.0^{a}	8±0.0^{a}	8.5±0.5^{b}	8.5±0.3^{b}	9±0.58^{c}	9±0.58^{c}	9.5±0.6^{d}	9.5±0.7^{d}
	30	8±0.0^{a}	8±0.0^{a}	8.5±0.3^{b}	8.5±0.5^{b}	9±0.55^{c}	9±0.55^{c}	9.5±0.5^{d}	9.5±0.5^{d}
	40	10±0.0^{a}	8±0.0^{c}	10.5±0.5^{a}	8.5±0.4^{c}	11±0.56^{b}	9±0.56^{d}	11.5±0.4^{b}	9.5±0.5^{d}
	50	12±0.0^{a}	8±0.0^{c}	12.5±0.6^{a}	8.5±0.5^{c}	13±0.50^{b}	9±0.56^{d}	13.5±0.5^{b}	9.5±0.4^{d}
	60	16±0.0^{a}	8±0.0^{c}	16.5±0.2^{a}	8.5±0.4^{c}	17±0.58^{b}	9±0.52^{d}	17.5±0.7^{b}	9.5±0.4^{e}
Leaf area (cm^2)	**0**	7.75±0.73^{a}	7.68±0.18^{a}	8.35±1.53^{a}	8.24±0.68^{a}	11.16±1.63^{b}	10.83±0.72^{b}	14.79±0.75^{c}	15.42±0.38^{c}
	10	9.14±0.74^{a}	9.01±0.18^{a}	9.74±1.56^{b}	9.63±0.71^{b}	12.52±1.64^{c}	12.16±0.73^{c}	16.16±0.75^{d}	16.75±0.38^{d}
	20	10.73±0.73^{a}	10.31±0.2^{a}	11.33±1.53^{b}	10.95±0.74^{b}	14.11±1.62^{c}	13.48±0.73^{c}	17.75±0.77^{d}	18.05±0.4^{d}
	30	12.59±0.73$_{a}$	10.58±0.18^{b}	13.19±1.53^{a}	11.22±0.71^{b}	15.97±1.61^{c}	13.75±0.73^{a}	19.66±0.74^{d}	18.36±0.39^{d}
	40	14.51±0.73^{a}	10.67±0.18^{d}	15.11±1.53^{a}	11.32±0.72^{d}	17.89±1.62^{b}	13.86±0.72^{a}	21.53±0.76^{c}	18.45±0.38^{e}
	50	17.07±0.74^{a}	10.7±0.18^{c}	17.68±1.53^{a}	11.34±0.72^{c}	20.45±1.62^{b}	13.88±0.72^{d}	24.08±0.75^{b}	18.47±0.38^{e}
	60	19.85±0.7^{a}	10.72±0.18^{d}	20.44±1.57^{a}	11.36±0.72^{d}	23.23±1.62^{b}	13.9±0.72^{d}	26.87±0.77^{c}	18.49±0.38^{e}

Note: *ww* - well water; *ws* - drought stress

3.3.2. Root growth of coffee plants in control and drought stress conditions

Drought stress negatively affects root traits in terms of root length, root number, and root volume. Those parameters were significantly lower for plants growing under drought stress than those growing under well-watered conditions. The impact of drought stress on the tested genotypes was also significantly different, and the highest and lowest root length, root number, and root volume were recorded in the relatively tolerant genotype of *Ca*74112 (16.33±1.53 cm, 40.15±1.55, 5.87±1.57 cm^3, respectively) and sensitive genotype of *Ca*754 (10.19±0.21 cm, 26.51±0.23, 3.15±0.21 cm^3, respectively), respectively (see Figures 3.1). As a result of drought stress, maximum and minimum increments of root length, number, and volume were identified in the relatively tolerant genotype of *Ca*74112 (77.15%, 78.11%, 78.25%, respectively), and *Ca*J-19 (57.26%, 56.05%, 58.36%, respectively), respectively (Appendix 7, 9 and 20).

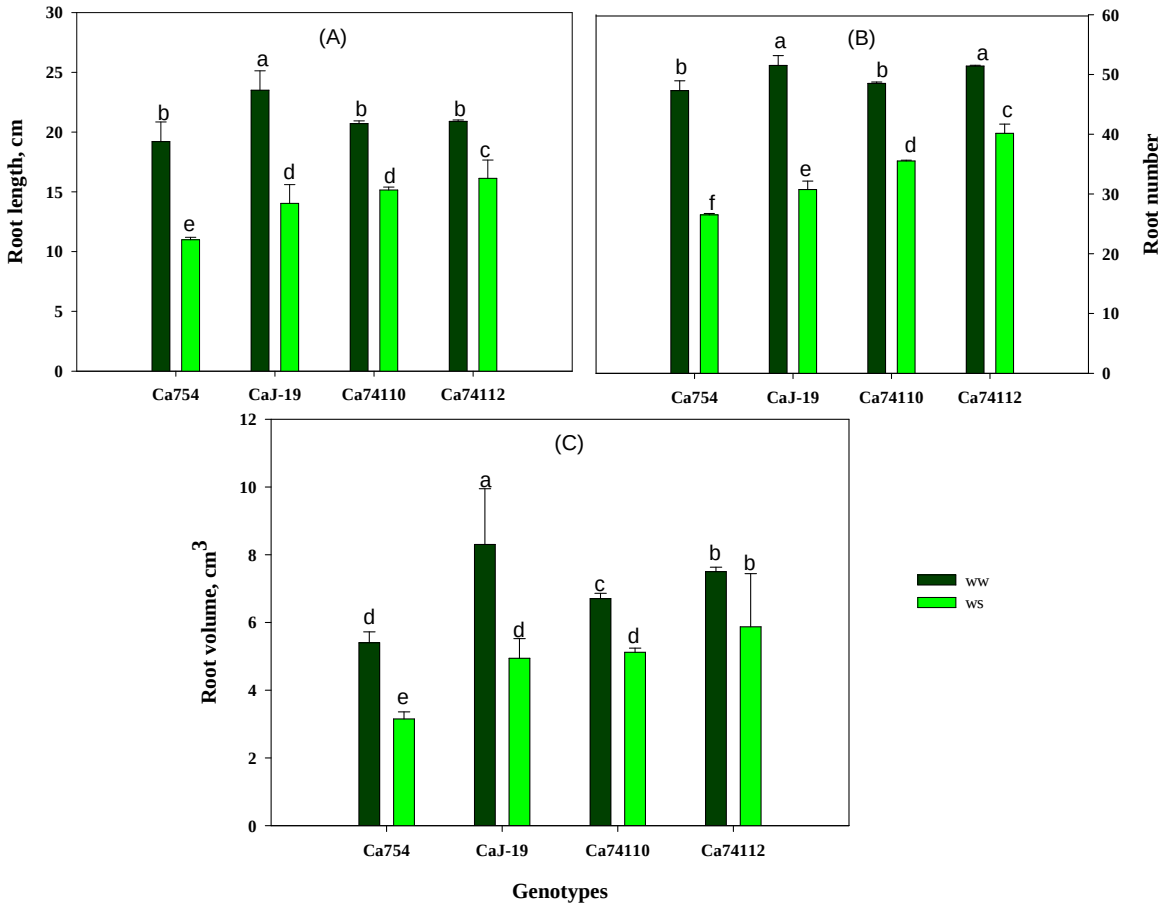

Figure 3.1. Root parameters of four *C. arabica* genotypes grown under well water (*ww*) and drought stress (*ws*) conditions, after 60 days of drought treatment begins: (**A**) root length (RL), (**B**) root number (RN), and (**C**) root volume (RV). Bars indicate mean±SD (*n*=15 replicates per genotype). Bars with the same letter do not differ significantly at $p<0.05$.

3.3.3. Biomass of coffee plants in control and drought stress conditions

At the end of the study, significantly lower fresh and dry weights were recorded in all genotypes grown under drought stress than in well-watered conditions. Under drought stress conditions, the highest and lowest root, leaf, stem, and total fresh mass were measured in the relatively tolerant genotype *Ca*74112 (3.03±0.48 g; 3.81±0.75 g; 2.46±1.12 g; 9.3 g, respectively) and sensitive genotype *Ca*754 (1.51±0.11 g; 1.63±0.26 g; 1.28±0.13 g; 4.43 g, respectively), respectively. Similarly, the highest and lowest root, leaf, stem, and total dry mass were measured in the

relatively tolerant genotype of *Ca*74112 (0.88±0.03 g; 1.11±0.04 g; 0.72±0.01 g; 2.71 g, respectively), and sensitive genotypes of *Ca*754 (0.4±0.03 g; 0.43±0.01 g; 0.34±0.06 g; 1.17 g, respectively), respectively (see Table 3.2) (Appendix 10 and 20).

Table 3.2. Mean variables of the fresh and dry weight of the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well water (*ww*) and drought stress (*ws*) conditions, after 60 days of the study. Symbols indicate mean±SD (n=15 replicates per genotype). Data in the same row with the same superscript are not significantly different at $p<0.05$.

Variable	*Ca*754		*Ca*J-19		*Ca*74110		*Ca*74112	
	ww	*ws*	*ww*	*ws*	*ww*	*ws*	*ww*	*ws*
RFM, g	4.12 ± 0.26^{a}	1.51 ± 0.11^{A}	4.65 ± 0.2^{b}	1.85 ± 0.06^{A}	4.15 ± 0.26^{a}	2.21 ± 0.06^{B}	4.23 ± 0.07^{a}	3.03 ± 0.48^{C}
LFM, g	4.38 ± 0.03^{a}	1.63 ± 0.26^{A}	5.45 ± 0.17^{b}	2.13 ± 0.06^{B}	4.67 ± 0.03^{a}	2.75 ± 0.06^{C}	5.25 ± 0.06^{b}	3.81 ± 0.75^{D}
SFM, g	3.2 ± 0.18^{a}	1.28 ± 0.13^{A}	4.3 ± 0.18^{b}	1.76 ± 0.06^{A}	3.3 ± 0.18^{a}	2.16 ± 0.06^{B}	4 ± 0.05^{b}	2.46 ± 1.12^{B}
TFM, g	11.7 ± 0.77^{a}	4.43 ± 0.08^{A}	14.4 ± 0.18^{b}	5.73 ± 0.07^{B}	12.12 ± 0.77^{a}	7.11 ± 0.07^{C}	13.48 ± 0.07^{b}	9.3 ± 0.1^{D}
RDM, g	1.09 ± 0.06^{a}	0.4 ± 0.03^{A}	1.28 ± 0.18^{a}	0.51 ± 0.06^{B}	1.17 ± 0.03^{a}	0.62 ± 0.07^{C}	1.23 ± 0.01^{a}	0.88 ± 0.03^{D}
LDM, g	1.16 ± 0.04^{a}	0.43 ± 0.01^{A}	1.49 ± 0.18^{b}	0.58 ± 0.06^{B}	1.32 ± 0.01^{b}	0.78 ± 0.06^{C}	1.53 ± 0.02^{b}	1.11 ± 0.04^{D}
SDM, g	0.84 ± 0.04^{a}	0.34 ± 0.06^{A}	1.18 ± 0.06^{b}	0.48 ± 0.06^{A}	0.93 ± 0.03^{a}	0.61 ± 0.06^{B}	1.16 ± 0.04^{b}	0.72 ± 0.01^{C}
TDM, g	3.09 ± 0.03^{a}	1.17 ± 0.06^{A}	3.95 ± 0.06^{a}	1.57 ± 0.07^{B}	3.42 ± 0.04^{a}	2.01 ± 0.05^{C}	3.92 ± 0.02^{a}	2.71 ± 0.03^{D}

Note: *ww* - well water; *ws* - drought stress; RFM – root fresh mass; LFM – leaf fresh mass; SFM – stem fresh mass; TFM – total fresh mass; RDM – root dry mass; LDM – leaf dry mass; SDM – stem dry mass; and, TDM – total dry mass.

3.3.4. Biomass allocation of coffee plants in control and drought stress conditions

Drought-stress effects on biomass allocation ratios differed among the genotypes, and in terms of root mass fraction (RMF), leaf mass fraction (LMF), stem mass fraction (SMF), dry matter content (DMC), and root-to-shoot ratio (R/S^{r}) genotypes under well-watered and drought-stressed conditions showed no significant ($p>0.05$) differences but minimum change of increasing and decreasing the mass fractions were recorded by the drought-stressed. Compared to well-watered, unlike the other genotypes, drought-stressed increased RMF, LMF, DMC and R/S^{r} of the genotype *Ca*74112 by 3.37%, 4.71%, 0.24% and 4.91%, respectively, but decreased the value of SMF by 10.22% while the other genotypes showed an increased SMF value. However, among the genotypes under drought-stressed conditions, significantly ($p<0.001$)

highest allocation of RMF is recorded in the genotype *Ca*754 (0.34±0.02 g g^{-1}), highest LMR in *Ca*74112 (0.41±0.04 g g^{-1}), highest SMF in *Ca*J-19 (0.31±0.03 g g^{-1}), highest DMC in *Ca*74112 (29.15%) and higher R/S ratio in *Ca*754 (0.52±0.08 g g^{-1}) (see Table 3.3).

3.3.5. Growth rate of coffee plants in control and drought stress conditions

Referring to the genotype's specific stem length (SSL), specific root length (SRL), leaf area ratio (LAR), and specific leaf area (SLA), a significant difference was recorded in genotypes between the well-watered and drought-stressed conditions, but not at a statistically significant level for *Ca*74112. However, the relatively tolerant genotypes showed significantly highest relative growth rate (RGR) under drought stress conditions, while all the genotypes showed similar responses of RGR under controlled condition.

Table 3.3. Mean variables of the biomass allocation and growth rate of the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well water (*ww*) and drought stress (*ws*) conditions, after 60 days of the study. Symbols indicate mean±SD (*n*=15 replicates per genotype). Data in the same row with the same superscript are not significantly different at $p<0.05$.

Variables	*Ca*754		*Ca*J-19		*Ca*74110		*Ca*74112	
	ww	*ws*	*ww*	*ws*	*ww*	*ws*	*ww*	*ws*
RMF, g g^{-1}	0.35±0.07^{a}	0.34±0.02^{a}	0.32±0.01^{a}	0.32±0.07^{a}	0.34±1.56^{a}	0.31±0.06^{a}	0.31±0.03^{a}	0.32±0.05^{a}
LMF, g g^{-1}	0.38±0.06^{a}	0.37±0.02^{a}	0.38±0.02^{a}	0.37±0.06^{a}	0.39±1.56^{a}	0.39±0.01^{a}	0.39±0.06^{a}	0.41±0.04^{b}
SMF, g g^{-1}	0.27±0.06^{a}	0.29±0.04^{a}	0.3±0.04^{b}	0.31±0.03^{b}	0.27±1.55^{a}	0.3±0.03^{b}	0.3±0.08^{b}	0.27±0.02^{a}
DMC, %	26.41±0.5^{a}	26.44±0.2^{a}	27.43±1.7^{a}	27.38±1.1^{a}	28.22±1.3^{b}	28.25±1.6^{b}	29.08±1.2^{b}	29.15±0.3^{b}
R/ S ratio, g g^{-1}	0.55±0.01^{a}	0.52±0.08^{b}	0.48±0.01^{c}	0.48±0.01^{c}	0.52±0.01^{b}	0.45±0.01^{d}	0.46±0.05^{d}	0.48±0.03^{c}
SSL, cm g^{-1}	27.56±2.3^{a}	33.47±2.1^{b}	21.25±1.5^{c}	27.6±1.4^{a}	29.26±2.3^{a}	25.16±1.7^{a}	25.35±0.8^{a}	25.19±1.1^{a}
SRL, cm g^{-1}	17.61±2.1^{a}	27.49±1.1^{b}	18.36±1.7^{a}	27.5±1.6^{b}	17.69±2.1^{a}	24.46±1.6^{b}	16.99±0.2^{a}	18.32±1.7^{a}
LAR, cm2 g^{-1}	6.42±0.74^{a}	9.16±0.42^{b}	7.45±0.11^{a}	7.24±0.13^{a}	6.79±0.74^{a}	6.91±1.62^{a}	6.85±1.62^{a}	6.82±0.15^{a}
SLA, cm2 g^{-1}	17.11±0.3^{a}	24.92±1.5^{b}	19.76±0.3^{a}	19.59±0.1^{a}	17.6±1.2^{a}	17.81±1.2^{a}	17.56±1.5^{a}	16.66±1.0^{a}
RGR, mg $g^{-1}d^{-1}$	13.61±2.1^{b}	11.49±1.1^{c}	13.36±1.7^{b}	14.5±1.6^{b}	14.69±2.1^{b}	18.46±1.6^{a}	14.99±0.2^{b}	19.32±1.7^{a}

Note: *ww* - well water; *ws* - drought stress; RMF – root mass fraction; LMF – leaf mass fraction; SMF – stem mass fraction; DMC – dry matter content; R/S ratio – root to shoot ratio; SSL – specific stem length; SRL – specific root length; LAR – leaf area ratio; and, SLA – specific leaf area, RGR – relative growth rate.

Unlike well-watered conditions, in drought-stressed conditions, significantly the highest SSL (p<0.001), LAR (p<0.05) and SLA (p<0.05) were recorded in the genotype *Ca*754 (33.47±2.31 cm g^{-1}; 9.16±0.42 cm^2 g^{-1}; 24.92±1.75 cm^2 g^{-1}, respectively), whereas the lowest SSL was recorded by the genotype *Ca*74110 (25.16±1.97 cm g^{-1}), and lowest LAR and SLA were recorded in the genotype *Ca*74112 (6.82±0.15 cm^2 g^{-1}; 16.66±1.05 cm^2 g^{-1}, respectively). In the meantime, under drought stress condition, the highest and lowest SRL (p<0.05) were recorded in the genotype *Ca*J-19 (27.50±1.46 cm g^{-1}) and *Ca*74112 (18.32±1.17 cm g^{-1}), respectively. Significantly (p<0.05), under drought stress condition, the highest RGR were recorded in the relatively tolerant genotypes of *Ca*74110 (18.46±1.6 mg $g^{-1}d^{-1}$) and *Ca*74112 (19.32±1.7 mg $g^{-1}d^{-1}$) (see Table 3.3).

3.3.6. Relative water content and stem water potential

Drought stress significantly (p<0.05) lowered relative water content (RWC) and water potential (Ψ_w,–Mpa). Under drought stress the mean RWC was higher in the relatively tolerant genotypes of *Ca*74112 (48.09±0.8%) and *Ca*74110 (43.4±0.29%), and a lower in the sensitive genotypes of *Ca*J-19 (32.57±0.13%) and *Ca*754 (30.24±0.21%). As a result of drought stress the minimum and maximum reduction of RWC under drought stress were recorded in the relatively tolerant genotypes of *Ca*74112 (41.89%) and *Ca*74110 (46.74%), and sensitive genotypes of *Ca*J-19 (60.32%) and *Ca*754 (62.74%), respectively (see Figure 3.2A).

In well-watered plants, the water potential of the genotypes throughout the study period was not significantly different (ranging between -1.44 to -1.48 Mpa). In plants grown in drought-stress conditions, prolonged exposure of plants to drought stress resulted in a significant decrease in the stem water potential. Under drought stress conditions, at the end of the experiment, the mean Ψ_w was significantly (p<0.001) dropped in all genotypes, and the higher Ψ_w were

maintained in the relatively tolerant genotypes of *Ca*74112 (-2.56±0.02, Mpa) and *Ca*74110 (-2.64±0.02, Mpa), whereas in the sensitive genotypes, the decrease was more apparent i.e. *Ca*J-19(-3.02±0.07, Mpa) and *Ca*754 (-3.11±0.02, Mpa). Fluctuations in Ψ_w revealed a contrasting behavior among the genotypes. As a result of drought stress, the percentage of the decrease in Ψ_w among the tested genotypes from highest to lowest were as follows *Ca*754 (53.24%), *Ca*J-19 (52.05%), *Ca*74110 (45.38%), and *Ca*74112 (42.14%) (see Figure 3.2B) (Appendix 11 and 20).

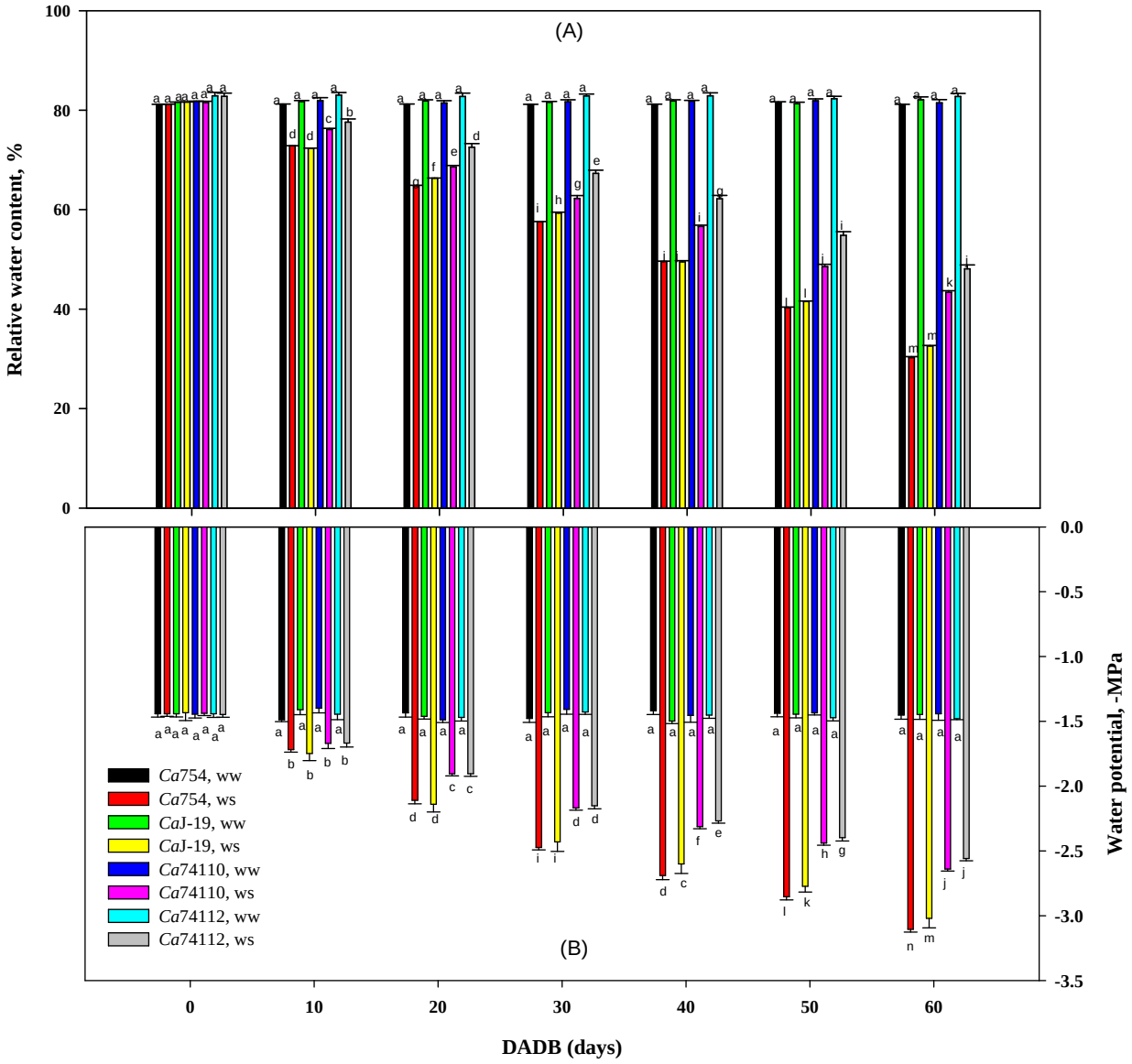

Figure 3.2. Effects of drought treatment on (**A**) relative water content (RWC), and (**B**) water potential of the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well water (*ww*) and drought stress conditions (*ws*). Bars indicate means±SD (*n*=3 replicates per genotype). Bars with the same letter do not differ significantly at $p<0.05$. DADB indicates the number of days after drought stress begins.

3.3.7. Gaseous exchanges, and water use efficiency

In plants grown under control conditions, throughout the study period, the CO_2 assimilation rate was not significantly different among the genotypes, with a range of 7.24 to 7.59 µmol $m^{-2}s^{-1}$. Under drought stress conditions the genotypes showed significantly different reductions in mean CO_2 assimilation rate. The higher A values were recorded in the relatively tolerant genotypes of *Ca*74112 (2.89±0.11 µmol $m^{-2}s^{-1}$) and *Ca*74110 (2.29±0.06 µmol $m^{-2}s^{-1}$), and the lower values in the sensitive genotypes *Ca*J-19 (1.55±0.13 µmol $m^{-2}s^{-1}$) and *Ca*754 (1.00±0.09 µmol $m^{-2}s^{-1}$). Comparing the reduction of CO_2 assimilation rate as a result of drought stress, significantly the highest rate of reduction was recorded in the sensitive genotype *Ca*754 (86.85%) whereas the lowest was in tolerant genotype *Ca*74112 (61.96%) (see Figure 3.3A). There were no significant differences in the stomatal conductance values when comparing plants grown under drought stress and well-watered conditions at the early stage, but at the end of the experiment, significant differences in Gs were displayed by the genotypes. Under drought stress, maximum Gs was recorded in the relatively tolerant genotypes *Ca*74112 (60.25±1.38 mmol $m^{-2}s^{-1}$), and *Ca*74110 (46.51±0.89 mmol $m^{-2}s^{-1}$), and the minimum Gs in the sensitive genotypes *Ca*J-19 (36.84±0.71 mmol $m^{-2}s^{-1}$) and *Ca*754 (30.28±0.86 mmol $m^{-2}s^{-1}$). As a result of drought stress the Gs decreased by 42.45%, 54.68%, 63.85%, and 70.24%, in the genotype *Ca*74112, *Ca*74110, *Ca*J-19, and *Ca*754, respectively (see Figure 3.3B). Similarly, the WUE showed a significant ($p<0.05$) reduction, to the end of the experiment, under drought stress conditions, but the tolerant genotypes of *Ca*74110 (2.41±0.03 mmol mol^{-1}) and *Ca*74112 (2.86±0.04 mmol mol^{-1}) had higher WUE than the sensitive genotypes *Ca*J-19 (2.07±0.02 mmol mol^{-1}) and *Ca*754 (1.84±0.07 mmol mol^{-1}).

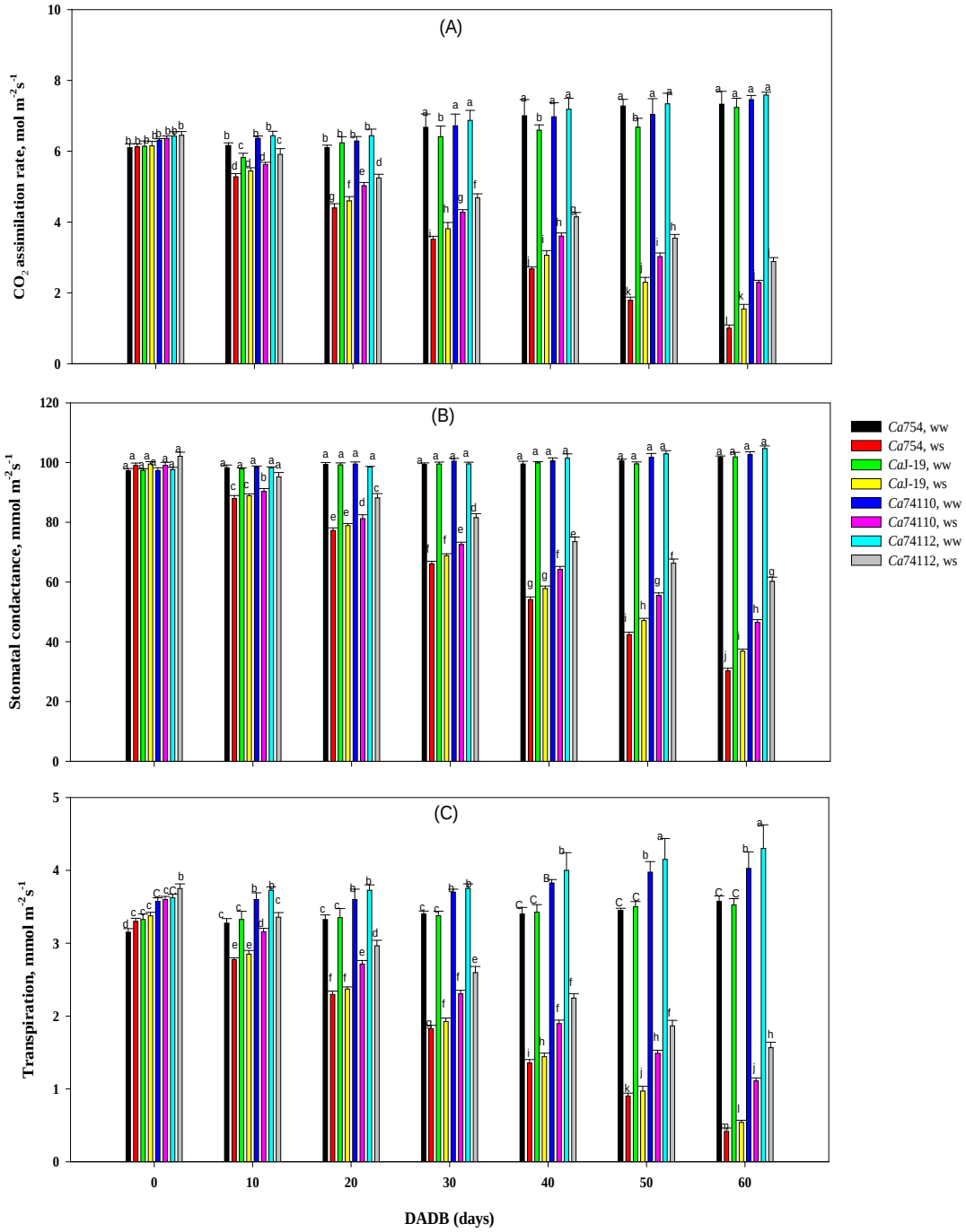

Figure 3.3. Effects of drought stress on (**A**) CO_2 assimilation rate, (**B**) stomatal conductance, and (**C**) transpiration rate of the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well water (*ww*) and drought stress conditions (*ws*). Bars indicate means±SD (n=15 replicates per genotype). Bars with the same letter do not differ significantly at $p<0.05$. DADB indicates the number of days after drought stress begins.

Under drought stress, at the end of the experiment, the higher transpiration rate was recorded in the relatively tolerant genotype *Ca*74112 (1.56±0.07 mmol $m^{-2}s^{-1}$) and *Ca*74110 (1.11±0.04 mmol $m^{-2}s^{-1}$), whereas the lower E value was displayed by the sensitive genotypes of *Ca*J-19 (0.54±0.03 mmol $m^{-2}s^{-1}$) and *Ca*754 (0.42±0.04 mmol $m^{-2}s^{-1}$). The minimum and maximum reduction of E as a result of drought stress were identified in the relatively tolerant genotypes of *Ca*74112 (63.6%), and *Ca*74110 (72.42%), and the sensitive genotypes of *Ca*J-19 (84.68%) and *Ca*754 (88.39%), respectively (see Figure 3.3C) (Appendix 11 and 20).

3.3.8. Photosynthetic pigments

Plants grown under well-watered conditions had significantly higher pigments content than those in drought-stress conditions. In drought stress, the result showed a significant decline of *Chl*-a (see Figure 3.4A), *Chl*-b (see Figure 3.4B), and total chlorophyll (see Figure 3.4C) content in all tested genotypes whereas in well-watered plants the amount of chlorophyll was relatively stable throughout the experiment. The highest and lowest *Chl*-a, *Chl*-b, and total chlorophyll content was detected in the relatively tolerant genotype *Ca*74112 (1.09, 0.41, and 1.5 mg g^{-1}fw, respectively) and *Ca*754 (0.63, 0.29, and 0.91 mg g^{-1}fw, respectively), respectively. The minimum and maximum reduction of *Chl*-a, content as a result of drought stress were identified in the relatively tolerant genotypes *Ca*74112 (40.27%) and *Ca*74110 (47.28%), and the sensitive genotypes *Ca*J-19 (52.44%) and *Ca*754 (56.96%), respectively. The maximum reduction of *Chl*-b content was identified in the sensitive genotype of *Ca*754 (21.92%). Similarly, the minimum and maximum reduction of total chlorophyll content were identified in the relatively tolerant genotypes *Ca*74112 (35.57%) and *Ca*74110 (41.51%), and the sensitive genotypes *Ca*J-19 (45.49%) and *Ca*754 (49.94%), respectively.

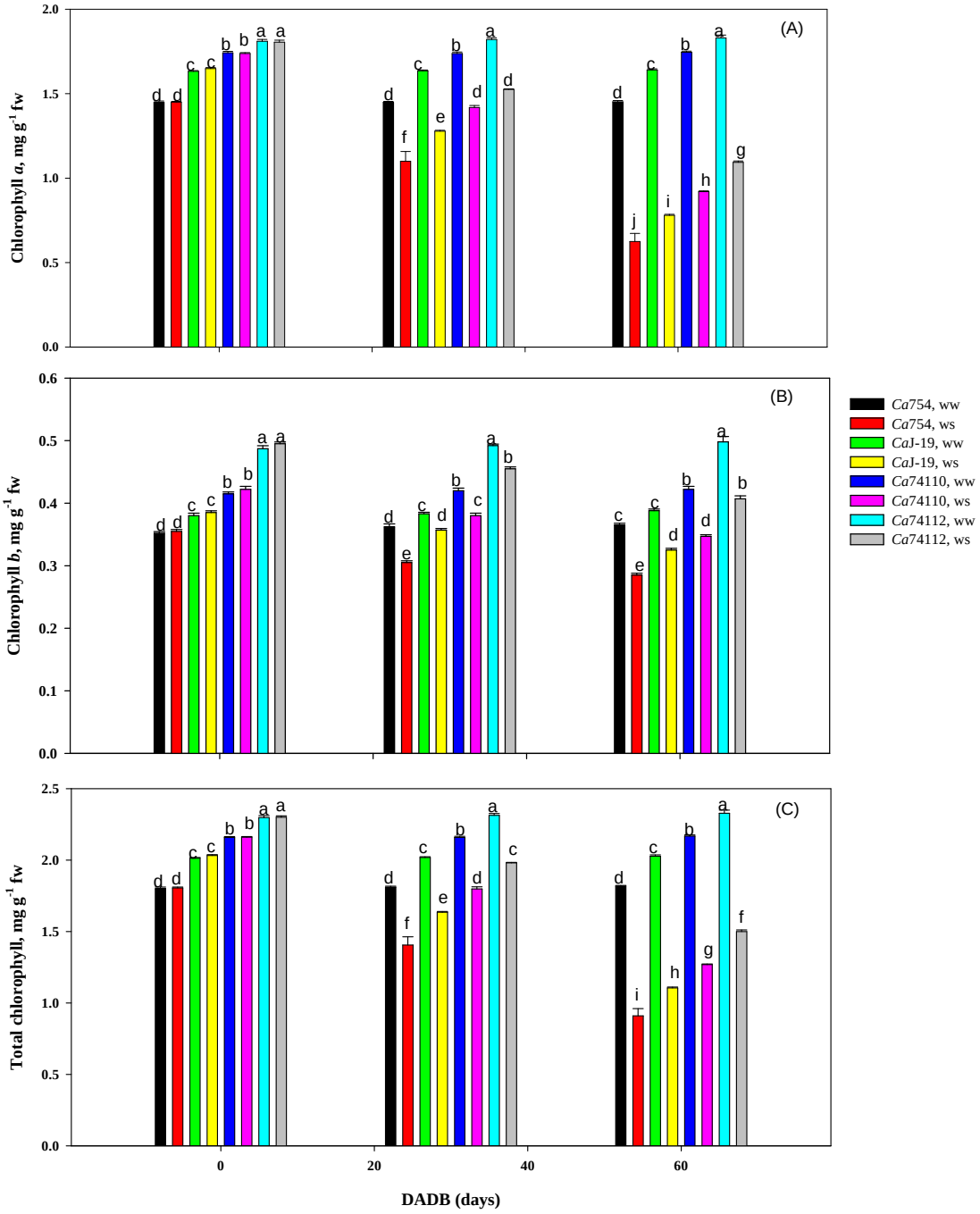

Figure 3.4. The effect of drought stress in (**A**) chlorophyll-a, (**B**) chlorophyll-b, and (**C**) total chlorophyll content of the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well-watered (*ww*) and drought stress conditions (*ws*). Bars indicate means±SD (n=15 replicates per genotype). Bars with the same letter do not differ significantly at $p<0.05$. DADB indicates the number of days after drought stress begins.

3.3.9. Stomatal densities

Significantly all the stomata were found on the lower leaf surface of the genotype. At the end of the experiment, stomatal densities in well-watered genotypes were significantly (p<0.05) higher than those under drought-stressed conditions. In the drought-stressed treated genotypes, the result showed that significantly (p<0.001) different and decreased stomatal densities, where the highest were observed in the genotype *Ca*74112 (275±3.19 mm^{-2}) followed by *Ca*74110 (245±1.67 mm^{-2}) and the lowest in the genotypes *Ca*754 (218.33±1.67 mm^{-2}) and *Ca*J-19 (223.33±1.93 mm^{-2}) (see Figure 3.5).

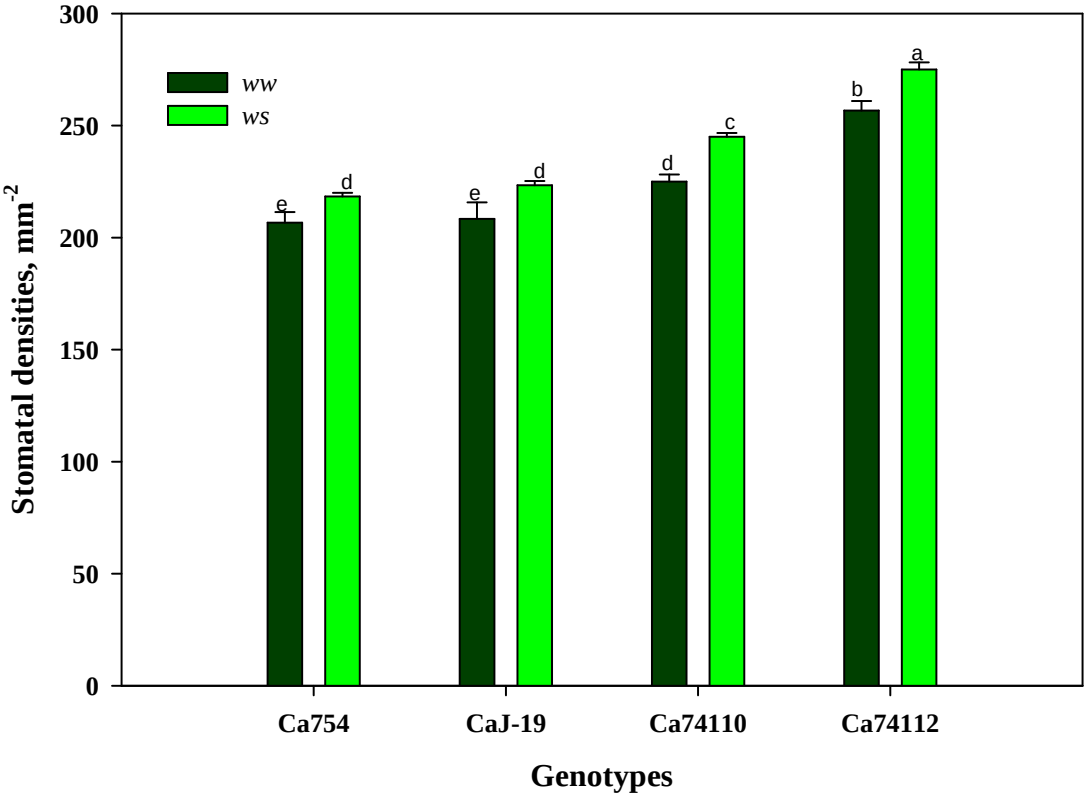

Figure 3.5. The effect of drought stress in stomatal densities of the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well-watered (*ww*) and drought stress conditions (*ws*). Bars indicate means±SD (n=15 replicates per genotype). Bars with the same letter do not differ significantly at p<0.05.

3.3.10. Cell membrane stability and relative cell injury

Significant differences were observed for the mean cell membrane stability (CMS) and relative cell injury (RCI) under drought stress conditions among the genotypes, at the end of the experiment. The highest mean CMS, an indication of stress tolerance, was observed in the relatively tolerant genotypes of *Ca*74112 (82.5±9.41%) and *Ca*74110 (73.31±7.32%), and the lowest CMS in the sensitive genotypes *Ca*754 (49.94±2.36%) and *Ca*J-19 (59.03±2.81%). Inversely, the highest and lowest mean RCI, an indication of stress sensitivity, were observed in the sensitive genotypes *Ca*754 (50.06±1.53%) and *Ca*J-19 (40.97±1.47%), and the relatively tolerant genotypes *Ca*74112 (17.5±4.48%) and *Ca*74110 (20.69±3.41%), respectively (see Figure 3.6).

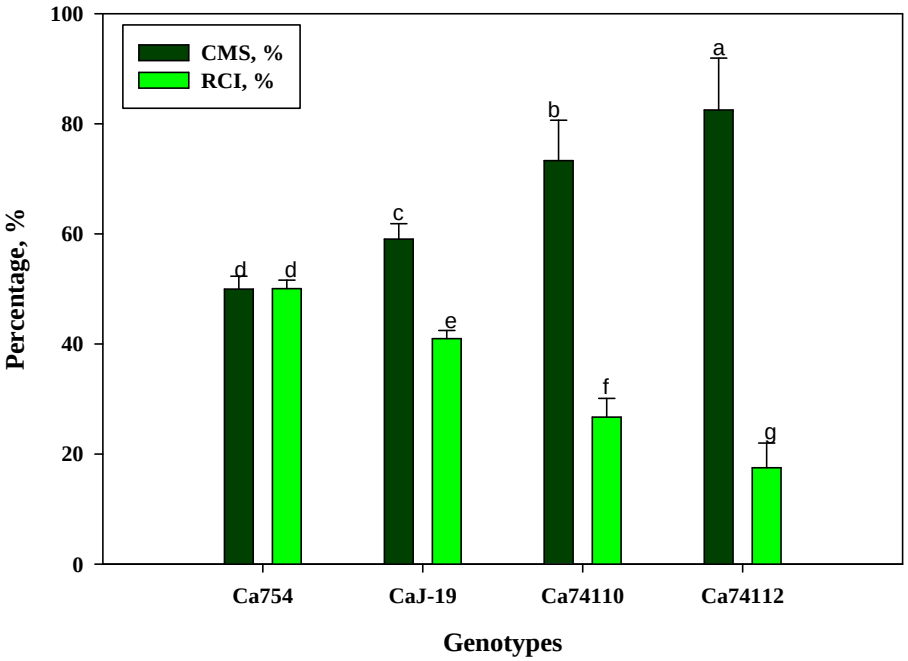

Figure 3.6. The CMS and RCI in the four adult coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well-watered (*ww*) and drought stress (*ws*) conditions, at the end of the 60 days experiment. Bars indicate means±SD (n=15 replicates per genotype). Bars with the same letter do not differ significantly at $p<0.05$.

3.3.11. Multivariate Analysis

3.3.11.1. PCA analysis

The eigenvalues and contribution rates of principal components were the basis for selecting principal components. The 31 vegetative and physiological indexes of the four genotypes were analysed by PCA. Two principal components were obtained, and their contribution rates were PC1-87.14% and PC2-10.53%, respectively, with a cumulative contribution rate of 97.67% (see Figure 3.7). Therefore, the first two principal components were selected as the important principal components of the drought resistance of the four coffee genotypes. There are a lot of vegetative and physiological factors with significant and higher loading correlation capacity in the PC1, which was related to a lot of vegetative growth, gas exchange, water parameters, stomatal densities, and cell membrane stabilities. However, PC2 was mainly weakly correlated and related to stem diameter, root mass fraction, leaf mass fraction, root-to-shoot ratio, specific stem length, specific leaf area and leaf area ratio (see Table 3.4).

To briefly assess the drought tolerance capacity of the genotypes, all growth and physiological parameters were taken in to consideration and analyzed, and the score value of PC1 and PC2 to the sum of the total PC values of the extracted principal components was taken as the weight. Based on this, the higher PC score values were positively correlated with the drought tolerant genotypes of *Ca*74112 (7.52 PCA score value) and *Ca*74110 (0.72 PCA score value), indicating more drought-stress tolerant, but also *Ca*74112 was more tolerant than *Ca*74110, which were followed by the sensitive genotypes of *Ca*J-19 (-4.1 PCA score value) and *Ca*754 (-4.2 PCA score value).

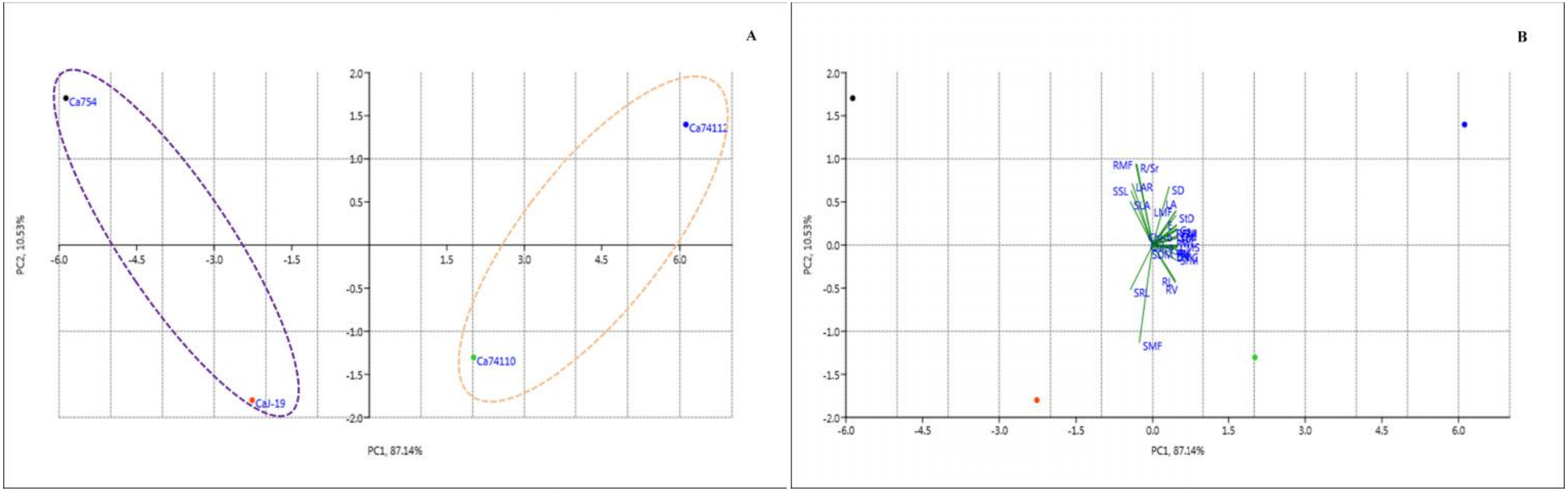

Figure 3.7. Principle component analysis (PCA) plot (x – first component, y – second component) plot indicating (**A**) biplot showing the clustering of genotypes, and (**B**) the row labels of each growth and physiological variables contributing to the separation, of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112.

Table 3.4. Eigenvalue and the cumulative contribution rate of each index of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under drought-stressed conditions. The coefficients table shows the linear combinations that make each principal component, and the color map shows the structure of the components. Values near zero (Red) indicate that a variable contributes little to the component, larger values (Green) indicate variables that contribute more to the component, whereas other colors indicate values in between red and green.

Measured index	Principal Component	
	PC1	PC2
Stem Length	0.198	0.043
Leaf Number	0.198	-0.01
Leaf Area	0.189	0.174
Root Length	0.189	-0.155
Root Number	0.198	0
Root Volume	0.184	-0.141
Root Fresh Mass	0.193	0.117
Leaf Fresh Mass	0.196	0.091
Stem Fresh Mass	0.197	-0.066
Root Dry Mass	0.193	0.116
Leaf Dry Mass	0.195	0.092
Stem Dry Mass	0.198	-0.041
Root to Shoot ratio	-0.126	0.425
Relative Water Content	0.193	0.02
Water potential	0.19	-0.031
Assimilation rate	0.198	-0.005
Root Mass Fraction	-0.125	0.427
Leaf Mass Fraction	0.19	0.148
Stem Mass Fraction	-0.11	-0.475
Specific Stem Length	-0.177	0.254
Specofic Root Length	-0.182	-0.224
Leaf Area Ratio	-0.169	0.271
Specific Leaf Area	-0.185	0.19
Stomatal conductance	0.196	0.081
Transpiration rate	0.194	0.082
Chlorophyll-a	0.198	0.007
Chlorophyll-b	0.195	0.064
Stomatal Densities	0.191	0.146
Cell Membrane Stability	0.197	-0.015

3.3.11.2. Pearson correlation analysis

To further understand the relationship between vegetative growth, water relation, gaseous exchange, pigments, stomatal densities and cell membrane stability, Pearson correlation was used to analyze the data. The study showed that, under drought-stressed conditions there was a positive correlation of water potential with stem length (r= 0.949), stem collar diameter (r= 0.798), leaf number (r= 0.960), leaf area (r= 0.905), root length (r= 0.892), root number (r= 0.964), root volume (r= 0.827), net assimilation rate (r= 0.974), stomatal conductance (r = 0.944) and transpiration rate (r= 0.973), chlorophyll-a (r= 0.950), chlorophyll-b (r= 0.903), total chlorophyll (r= 0.943), stomatal densities (r= 0.931) and cell membrane stability (r= 0.981). Furthermore, the study showed that a strong positive correlation (r= 0.995) between the leaf's relative water content and water potential was exited. Stomatal conductance was positively correlated with net assimilation rate (r= 0.988) and transpiration rate (r= 0.989). Relative growth rate was positively correlated with net assimilation rate (r= 0.976) and water use efficiency (r= 0.954). However, water potential was negatively correlated with root-to-shoot ratio (r= -0.706), specific stem length (r= -0.856), specific root length (r= -0.873), specific leaf area (r= -0.872), leaf area ratio (r= -0.783), root mass fraction (r= -0.703) and stem mass fraction (r= -0.480). The correlation of stomatal densities with specific leaf area (r= -0.804), and leaf area ratio (r= -0.685) was significantly negative (see Figure 3.8).

r	SD	LN	LA	RL	RN	RV	RFM	LFM	SFM	RDM	LDM	SDM	DMC	R/S ratio	SSL	SRL	LAR	SLA	RWC	Ψw	A	Gs	E	Chl-a	Chl-b	StD	CMS	RMF	LMF	SMF
SL	0.676	0.996	0.972	0.932	0.997	0.910	0.990	0.996	0.982	0.990	0.996	0.989	0.995	-0.577	-0.857	-0.945	-0.818	-0.906	0.971	0.949	0.994	0.998	0.984	0.998	0.992	0.980	0.990	-0.569	0.973	-0.617
SD	-	0.651	0.754	0.445	0.669	0.340	0.676	0.688	0.610	0.668	0.696	0.632	0.638	-0.291	-0.376	-0.773	-0.251	-0.402	0.805	0.798	0.692	0.707	0.797	0.643	0.602	0.773	0.706	-0.296	0.804	-0.638
LN	0.651	-	0.946	0.958	1.000	0.933	0.973	0.984	0.995	0.973	0.984	0.999	1.000	-0.649	-0.900	-0.912	-0.862	-0.938	0.972	0.960	0.998	0.987	0.974	0.999	0.983	0.959	0.996	-0.642	0.953	-0.541
LA	0.754	0.946	-	0.821	0.952	0.801	0.991	0.987	0.910	0.989	0.988	0.928	0.942	-0.385	-0.711	-0.996	-0.661	-0.782	0.944	0.905	0.950	0.986	0.979	0.954	0.968	0.998	0.942	-0.378	0.995	-0.781
RL	0.445	0.958	0.821	-	0.951	0.989	0.883	0.901	0.979	0.885	0.899	0.970	0.963	-0.783	-0.982	-0.764	-0.971	-0.998	0.888	0.892	0.947	0.904	0.872	0.954	0.926	0.840	0.945	-0.774	0.827	-0.308
RN	0.669	1.000	0.952	0.951	-	0.924	0.976	0.986	0.993	0.975	0.986	0.997	0.999	-0.640	-0.891	-0.919	-0.850	-0.930	0.977	0.964	0.999	0.990	0.980	0.999	0.983	0.965	0.997	-0.633	0.960	-0.555
RV	0.340	0.933	0.801	0.989	0.924	-	0.873	0.885	0.952	0.877	0.881	0.944	0.939	-0.719	-0.961	-0.744	-0.969	-0.987	0.832	0.827	0.914	0.882	0.829	0.933	0.923	0.812	0.906	-0.708	0.792	-0.320
RFM	0.676	0.973	0.991	0.883	0.976	0.873	-	0.998	0.947	1.000	0.998	0.960	0.971	-0.457	-0.785	-0.976	-0.749	-0.851	0.945	0.910	0.970	0.996	0.975	0.980	0.993	0.990	0.962	-0.448	0.982	-0.715
LFM	0.688	0.984	0.987	0.901	0.986	0.885	0.998	-	0.962	0.998	1.000	0.973	0.982	-0.504	-0.812	-0.968	-0.772	-0.871	0.960	0.930	0.982	0.999	0.983	0.989	0.994	0.990	0.976	-0.496	0.983	-0.682
SFM	0.610	0.995	0.910	0.979	0.993	0.952	0.947	0.962	-	0.947	0.961	0.999	0.996	-0.717	-0.938	-0.867	-0.904	-0.965	0.961	0.957	0.992	0.967	0.953	0.992	0.966	0.928	0.992	-0.710	0.921	-0.456
RDM	0.668	0.973	0.989	0.885	0.975	0.877	1.000	0.998	0.947	-	0.998	0.960	0.971	-0.457	-0.787	-0.974	-0.752	-0.854	0.942	0.906	0.970	0.995	0.973	0.981	0.994	0.988	0.961	-0.448	0.980	-0.713
LDM	0.696	0.984	0.988	0.899	0.986	0.881	0.998	1.000	0.961	0.998	-	0.973	0.982	-0.504	-0.810	-0.969	-0.768	-0.868	0.962	0.933	0.983	1.000	0.985	0.989	0.992	0.991	0.977	-0.496	0.985	-0.683
SDM	0.632	0.999	0.928	0.970	0.997	0.944	0.960	0.973	0.999	0.960	0.973	-	0.999	-0.688	-0.921	-0.889	-0.885	-0.954	0.968	0.961	0.996	0.977	0.965	0.996	0.975	0.943	0.995	-0.681	0.937	-0.495
DMC	0.638	1.000	0.942	0.963	0.999	0.939	0.971	0.982	0.996	0.971	0.982	0.999	-	-0.655	-0.906	-0.907	-0.870	-0.944	0.968	0.956	0.997	0.985	0.971	0.999	0.984	0.955	0.995	-0.647	0.948	-0.532
R/S ratio	-0.291	-0.649	-0.385	-0.783	-0.640	-0.719	-0.457	-0.504	-0.717	-0.457	-0.504	-0.688	-0.655	-	0.882	0.303	0.853	0.812	-0.639	-0.706	-0.653	-0.526	-0.546	-0.622	-0.518	-0.438	-0.671	1.000	-0.442	-0.271
SSL	-0.376	-0.900	-0.711	-0.982	-0.891	-0.961	-0.785	-0.812	-0.938	-0.787	-0.810	-0.921	-0.906	0.882	-	0.642	0.990	0.991	-0.833	-0.856	-0.890	-0.820	-0.795	-0.889	-0.840	-0.740	-0.892	0.874	-0.729	0.129
SRL	-0.773	-0.912	-0.996	-0.764	-0.919	-0.744	-0.976	-0.968	-0.867	-0.974	-0.969	-0.889	-0.907	0.303	0.642	-	0.589	0.720	-0.919	-0.873	-0.917	-0.966	-0.962	-0.922	-0.943	-0.989	-0.909	0.296	-0.988	0.835
LAR	-0.251	-0.862	-0.661	-0.971	-0.850	-0.969	-0.749	-0.772	-0.904	-0.752	-0.768	-0.885	-0.870	0.853	0.990	0.589	-	0.984	-0.764	-0.783	-0.844	-0.776	-0.732	-0.854	-0.816	-0.685	-0.843	0.844	-0.668	0.087
SLA	-0.402	-0.938	-0.782	-0.998	-0.930	-0.987	-0.851	-0.871	-0.965	-0.854	-0.868	-0.954	-0.944	0.812	0.991	0.720	0.984	-	-0.863	-0.872	-0.926	-0.875	-0.841	-0.932	-0.900	-0.804	-0.924	0.803	-0.790	0.247
RWC	0.805	0.972	0.944	0.888	0.977	0.832	0.945	0.960	0.961	0.942	0.962	0.968	0.968	-0.639	-0.833	-0.919	-0.764	-0.863	-	0.995	0.984	0.970	0.991	0.966	0.933	0.964	0.988	-0.635	0.969	-0.564
Ψw	0.798	0.960	0.905	0.892	0.964	0.827	0.910	0.930	0.957	0.906	0.933	0.961	0.956	-0.706	-0.856	-0.873	-0.783	-0.872	0.995	-	0.974	0.944	0.973	0.950	0.903	0.931	0.981	-0.703	0.939	-0.480
A	0.692	0.998	0.950	0.947	0.999	0.914	0.970	0.982	0.992	0.970	0.983	0.996	0.997	-0.653	-0.890	-0.917	-0.844	-0.926	0.984	0.974	-	0.988	0.983	0.996	0.976	0.964	0.999	-0.646	0.961	-0.546
Gs	0.707	0.987	0.986	0.904	0.990	0.882	0.996	0.999	0.967	0.995	1.000	0.977	0.985	-0.526	-0.820	-0.966	-0.776	-0.875	0.970	0.944	0.988	-	0.989	0.991	0.990	0.991	0.983	-0.519	0.986	-0.668
E	0.797	0.974	0.979	0.872	0.980	0.829	0.975	0.983	0.953	0.973	0.985	0.965	0.971	-0.546	-0.795	-0.962	-0.732	-0.841	0.991	0.973	0.983	0.989	-	0.974	0.959	0.990	0.983	-0.541	0.993	-0.658
Chl-a	0.643	0.999	0.954	0.954	0.999	0.933	0.980	0.989	0.992	0.981	0.989	0.996	0.999	-0.622	-0.889	-0.922	-0.854	-0.932	0.966	0.950	0.996	0.991	0.974	-	0.990	0.964	0.992	-0.614	0.957	-0.566
Chl-b	0.602	0.983	0.968	0.926	0.983	0.923	0.993	0.994	0.966	0.994	0.992	0.975	0.984	-0.518	-0.840	-0.943	-0.816	-0.900	0.933	0.903	0.976	0.990	0.959	0.990	-	0.969	0.967	-0.508	0.957	-0.644
StD	0.773	0.959	0.998	0.840	0.965	0.812	0.990	0.990	0.928	0.988	0.991	0.943	0.955	-0.438	-0.740	-0.989	-0.685	-0.804	0.964	0.931	0.964	0.991	0.990	0.964	0.969	-	0.960	-0.431	0.999	-0.746
CMS	0.706	0.996	0.942	0.945	0.997	0.906	0.962	0.976	0.992	0.961	0.977	0.995	0.995	-0.671	-0.892	-0.909	-0.843	-0.924	0.988	0.981	0.999	0.983	0.983	0.992	0.967	0.960	-	-0.665	0.958	-0.529
RMF	-0.296	-0.642	-0.378	-0.774	-0.633	-0.708	-0.448	-0.496	-0.710	-0.448	-0.496	-0.681	-0.647	1.000	0.874	0.296	0.844	0.803	-0.635	-0.703	-0.646	-0.519	-0.541	-0.614	-0.508	-0.431	-0.665	-	-0.436	-0.277
LMF	0.804	0.953	0.995	0.827	0.960	0.792	0.982	0.983	0.921	0.980	0.985	0.937	0.948	-0.442	-0.729	-0.988	-0.668	-0.790	0.969	0.939	0.961	0.986	0.993	0.957	0.957	0.999	0.958	-0.436	-	-0.744

Figure 3.8. Pearson correlation (***r***) analysis among the growth and physiological parameters of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under drought-stressed conditions.

3.4. Discussion

3.4.1. The extent of drought stress impact on growth varies among coffee genotypes

Drought stress is well known to reduce the growth and physiology of *C. arabica* (Dias *et al*., 2007). Under drought, there is a decline in turgor pressure that leads to a reduction in cell division, elongation, and expansion, which then decreases growth and development, gas exchange, morphological, molecular, and other biochemical activities (Leon-Burgos *et al*., 2022). In this study, the result showed that the impact of drought stress varies among the genotypes, and yet it reduced the growth of shoot, root, biomass, water relations, gas exchange, chlorophyll pigments, cell membrane stability, and increased stomatal densities, and relative cell injury.

Water stress is one of the environmental factors that impose drastic effects on the growth and development of crop plants, and *C. arabica* is comparatively more sensitive to water stress conditions throughout the whole growth stages (DaMatta *et al*., 2007). The present study showed a significant reduction in terms of shoot growth and development identified when the coffee genotypes were grown under drought stress than the well-watered conditions. However, the study revealed that, by withstanding the drought stress, the relatively tolerant genotypes of *Ca*74110 and *Ca*74112, showed much better efficiency in terms of key shoot growth and developmental indicators such as height, collar diameter, leaf area, and leaf number than the sensitive genotypes of *Ca*754 and *Ca*J-19. Shao *et al*. (2009) stated that the impact of drought stress usually depends on the intensity, severity, duration, genotype, and as well growth stage of the plant. According to Oguz *et al*. (2022), a shortage of water content or turgor pressure, disrupts the cellular mitosis process that greatly restricts and reduces cell division, cell elongation, and differentiation, and consequently limits the growth and development of a plant's

shoot as well as root systems. Cai *et al*. (2007) also forwarded that, drought stress leads to disturbing the plant water relations, decreasing leaf initiation rate, and causing physiological injuries in the tissue. Similar to this study, Tounekti *et al*. (2018) reported that the minimum and maximum growth reduction impact of drought stresses in stem height and diameter, and leaf number and area are caused by suppressing cell division and elongation. Tavares-Junior *et al*. (2002) and Gheidary *et al*. (2017) reported the reduction of shoot height, collar diameter, leaf area, and disruption and abortion of buds and flowers under drought stress conditions. Findings from Razmjoo *et al*. (2008) stated that drought stress reduced the entry of macro and micronutrients into the plant leading to a reduction in shoot length. The development of sufficient leaf number and leaf area is vital for the coffee plants to undertake the process of photosynthesis effectively which has a great impact on the growth and development (Kapoor *et al*., 2020). The lower the leaf area and number, the lower the photosynthetic rate, and the less catabolic and anabolic biochemical reactions to supply the macro- and micro-molecules needed for the growth and development of a plant (Taiz and Zeiger, 2010). Similar to this study, Bhargavi *et al*. (2017) reported a reduction in the number of leaves when *Andrographis paniculate* was subjected to drought stress. Srivastava and Srivastava (2014) also reported the declining leaf area in *Petroselinum crispum* L. and *Stevia rabaudiana* when grown under drought-stress conditions. Furthermore, Shao *et al*. (2009) also stated that, apart from the reduction in the number of leaves and leaf sizes, an increase in leaf senescence is as well a consequence of drought stress.

The roots, one of the most sensitive parts of coffee plants, play a key role in the acquisition of water and nutrients, provide structural support, ensure tolerance against abiotic stresses, and regulate rhizosphere zone and absorption by symbiotic associations with other microorganisms

(Smika and Klute, 1982). In the present study, drought stress influenced the growth and development of the four coffee genotype's root systems, but the impact varies among the genotypes. The reductions in root growth are attributed to a decrease in root number and length, which ultimately decreases the volume of the root system. Under the drought stress experiment, the relatively tolerant genotypes *Ca*74112 and *Ca*74110 displayed better root length, number, and volume, compared with the sensitive genotypes of *Ca*754 and *Ca*J-19, indicating more water conservation characteristics to tolerate the drought stress, therefore, maintaining a more favorable internal water status.

According to Wright *et al*. (1999), roots are the first signaling parts of the plant to recognize the availability of soil water content, which then the plant promotes the adjustment of the root's growth and development in terms of type, length, amount, volume growth, and organization characteristics. Besides, according to Tagliavini *et al*. (1993) and Hussain *et al*. (2020), reported that drought-tolerant genotypes develop deeper and more developed root systems to support the plants to acclimatize to a wide range of drought stress conditions. According to Dias *et al*. (2007), when tolerant genotypes are grown under water deficit conditions, developmental changes and maximization of root number, length, density, volume, size, and diameter are shown more than sensitive genotypes, which facilitate plant adaptation to drought conditions. Similar to this study, Osakabe *et al*. (2014), and Smith and De Smet (2012) reported the escalation of root length and stated the elongation of the root is a vital strategy to maximize the retention of soil water content, and nutrient absorption to improve the plant root to shoot proportion, and subsequently reducing the plant biomass. Under drought stress, those genotypes with long and advanced root systems can easily uptake water and nutrients and have successful plant growth and development by avoiding drought stress (Ronchi *et al*., 2007; Mateva *et al*., 2022).

Furthermore, Vurayai *et al*. (2011), showed the impact of healthy roots in promoting effective growth and development.

As a result of the reduction of shoot and root growth and development under drought stress conditions, the reduction of fresh biomass, i.e., stem fresh mass, leaf fresh mass, root fresh mass, and total fresh mass, and dry biomass, i.e., stem dry mass, leaf dry mass, root dry mass, and total dry mass were computed, and the values (g) in the relatively tolerant genotypes of *Ca*74112 followed by *Ca*74110 were significantly higher than the sensitive genotypes of *Ca*754 and *Ca*J-19. This decrease of total fresh mass in the four genotypes is highly correlated with the reduction of stem height (r= 0.999), leaf number (r= 0.992), leaf area (r= 0.978), root length (r= 0.922), root number (r= 0.993), and root volume (r= 0.905). The reduction of the dry mass is highly correlated (r= 1.00) with the fresh mass of the coffee genotypes in the experiment. The reduction of biomass under ***ws*** conditions in four of the genotypes was in line with the results obtained by Dias *et al*. (2007) and Poorter *et al.* (2011).

According to Taiz and Zeiger (2010), plants under drought stress usually exhibit a substantial decline in plant biomass, fresh and dry biomass. DaMatta and Ramalho (2006) reported the reduction of fresh and consequently dry weight in coffee plants when they were subjected to drought stress. Similarly, DaMatta (2004) reported a significant reduction of fresh weight in *C. arabica* plants under drought stress, suggesting that the species is sensitive to water stress. Dias *et al*. (2007) reported that under drought, a decrease in dry mass in drought-tolerant (Siriema) and drought-sensitive (Catucai) *C. arabica* genotypes, but the tolerant ones with more mass than the sensitive genotypes. Poorter *et al*. (2011) and Soizig *et al*. (2021) carried out drought impact on various growth parameters, and reported a significant reduction in fresh and dry mass of the plants under study.

It is often reported that plants grown under a limited water supply show a shift in biomass allocation from leaves and stems to roots (Poorter *et al*., 2011). In this study, except the genotype *Ca*74112, allocation to roots remained unaffected in the other genotypes. Under drought-stressed conditions the biomass allocation in the relatively tolerant genotype *Ca*74112 increased in the root (root mass fraction), and root-to-shoot ratio, but decreased in stem (stem mass fraction) and had no change in terms of leaf mass fraction, an indication of tolerance of water stress (Schenk, 2006). Under drought-stressed conditions, the sensitive genotypes of *Ca*J-19 and *Ca*754 decreased in root mass fraction, leaf mass fraction, and root-to-shoot ratio, while the stem mass fraction increased, where the result is an indication of sensitivity towards water stress (Poorter and Navas, 2003). Similarly, dry matter content of the relatively tolerant genotypes *Ca*74112 and *Ca*74110 increased under drought-stressed than well-watered conditions.

Our result showed that the relatively tolerant genotype *Ca*74112 increased root development at the expense of shoot mass while growing under drought-stressed conditions, indicating the genotype can better explore water from the soil. This is consistent with a previous study that found higher root mass fraction and lower leaf mass fraction and stem mass fraction, where the stem transports water and nutrients from the root to other parts of the plant and provides key support for leaves and other parts of the plant, under water scarcity conditions (Pour-Aboughadareh *et al*., 2019). The leaf, as another major outlet of water via transpiration, is a pivotal component of photosynthesis (Eziz *et al*., 2017). Under drought stress, plants would invest less in the stem and leaf to reduce the water loss to the minimum level (Lambers *et al*., 2008). Under drought-stressed conditions, differences in specific stem length, specific root length, leaf area ratio, and specific leaf area among the genotypes were mostly associated with the water potential status than with morphological adjustments, which is in accordance with Dias

et al. (2007). This is the main reason that the relatively tolerant genotype *Ca*74112 has a higher proportion of root mass fraction and root-to-shoot ratio, which ensures the better acquisition of water and nutrients to support higher biomass accumulation (Kleyer *et al*., 2018). The drought-induced decreases in relative growth rate were mostly dependent on decreases in CO_2 assimilation rate. These results are in agreement with Poorter *et al*. (2011) and Soizig *et al*. (2021), which pointed out that decreases in relative growth rate due to drought were caused primarily by changes in assimilation and, to a lesser extent, by changes in specific leaf area.

3.4.2. Relative water contents and water potential are affected differently among genotypes under drought stress

The relative water content is a good reference for the water conditions of the plant as it represents the balance between water supply and transpiration (Pirzad *et al*., 2011). Drought stress leads to a decrease in plants' relative water content and water potential (Hayatu *et al*., 2014). The result of the current study revealed that under well-watered conditions all the genotypes possessed significantly similar relative water content throughout the experiment ranging between 80-and 84%. However, the responses of the RWC under drought-stressed conditions in the four genotypes are significantly lower and range between 30-49%. Moreover, among the genotypes under ***ws*** conditions, significantly the highest relative water content is recorded by the relatively tolerant genotype *Ca*74112 (41.89% reduction), while the lowest was recorded in the sensitive genotype *Ca*J-19 (60.32% reduction). In this study, under extreme drought stresses, water potential in all the genotypes, decreased to different degrees, indicating that the genotypes could absorb more water by reducing water potential to resist drought stress. Among them, the relatively tolerant genotype *Ca*74112 (reduction in 42.14 Ψ_w)

and sensitive genotype *Ca*754 (reduction in 53.24 Ψ_w) decreased the least and most under well-watered, respectively.

Based on Barrs and Weatherley (1962), leaves with relative water content between 30 and 40% reveal plants under water scarcity. According to da Silva *et al*. (2022) when relative water content values around 98%, it refers to leaves turgidity. It is known that smaller reductions in water potential correlate with higher water retention capacity and adaptation to drought stress (Pirzad *et al*., 2011). The difference in the decline of water status among the four genotypes in different degrees is related to their unique stomatal control and cuticular transpiration rate (DaMatta and Ramalho, 2006).

These results indicate that the relatively tolerant genotypes of *Ca*74112 and *Ca*74110 can maintain a higher water balance than the sensitive genotypes *Ca*J-19 and *Ca*754. The leaves of *Ca*74112 and *Ca*74110 were waxier than the leaves from the other genotypes, which would be beneficial for reducing water transpiration during drought stress. Similar to this study, Soltys-Kalina *et al*. (2016) reported the reduction of the relative water content of potatoes under drought stress conditions, but the report also identified that the tolerant genotypes, like *Ca*74110 and *Ca*74112, are characterized by having higher relative water contents than the sensitive genotypes.

Genotypic variation of water potential may be attributed to differences in the ability to absorb more water from the soil and the ability to reduce water loss through stomata (Siddique *et al*., 2000). It may also be due to differences in the ability of genotypes to maintain tissue turgor and hence physiological activities (Terzi and Kadioglu, 2006). At the cell level, plants attempt to decrease the damaging effects of stress by altering their metabolism to cope with stress.

Genotypes that maintain higher relative water content and water potential under drought stress are believed to be more tolerant and give higher growth and development than others (Bayomi *et al*., 2008).

The results herein were similar to those from studies performed in *C. arabica* by Dias *et al.* (2007) and Tounekti *et al*. (2018), where sensitive genotypes like *Ca*J-19 and *Ca*754 with lower RWC and water potential, leads to a decrease in the photosynthetic potential, which confirmed the lower efficiency of CO_2 assimilation, which in turn resulted in lower growth performance under drought-stressed conditions due to water limitation. The genotype having higher root length and volume, like the relatively tolerant genotypes of, *Ca*74112 and *Ca*74110, under drought stress, should have high relative water content and water potential (Lugojan and Ciulca, 2011). Under low water potential status, the cell membrane is subjected to changes such as an increase in penetrability and a decrease in sustainability (Blokhina *et al*., 2003). Microscopic investigations of dehydrated cells revealed damages, including cleavage in the membrane and sedimentation of cytoplasm content (Blackman *et al*., 2009).

3.4.3. Drought stress affected the gas exchange differently in coffee genotypes

Gas exchange parameters such as photosynthesis, transpiration, and stomatal conductance are key indicators of water shortage in plants and are useful for evaluating the tolerance responses of genotypes (DaMatta *et al*., 2003; Bhusal *et al*., 2021). With the increasing intensity of drought stress, plants are subjected to the reduction of photosynthesis assimilation rate, stomatal conductance, and transpiration rate by influencing the indices of gaseous exchange parameters (Taiz and Zeiger, 2010). It is well known that drought significantly affects the whole activity of

physiology as water is vital for various biochemical processes, and the reduction of leaf photosynthesis and gas exchange under water stress (Pinheiro *et al*., 2005; Tombesi *et al*., 2015).

As observed in this study, there was a strong relationship between the water potential reduction in the genotypes, and the responses of net assimilation rate, stomatal conductance, and transpiration rate. Stomatal conductance was positively correlated with net assimilation rate (r= 0.988) and transpiration rate (r= 0.989), and stomatal conductance is also strongly correlated (r= 0.986) with transpiration rate. Throughout the experiment, under well-watered conditions, all the genotypes had a more or less constant net assimilation rate, stomatal conductance, and transpiration rate which range between 6-8 µmol m^{-2} s^{-1}, 90-105 mmol m^{-2} s^{-1}, and 3-5 mmol m^{-2} s^{-1}. The net assimilation rate, stomatal conductance, and transpiration rate values of the four genotypes decreased as drought-stress was prolonged. As the leaf water potential decreased, the A decreased significantly in all genotypes. As the drought-stress intensified, the CO_2 assimilation rate in the sensitive genotypes of *Ca*754 (A reduced by 86.35%, than well-watered) and *Ca*J-19 (A reduced by 78.65%, than well-watered) decreased more than in the relatively tolerant genotype of *Ca*74112 (A reduced by 61.96%, than well-watered) and *Ca*74110 (A reduced by 69.24%, than well-watered). The respective values suggest that the photosynthetic mechanism in *Ca*J-19 and *Ca*754 is more sensitive to water stress than *Ca*74112 and *Ca*74110.

The lower water availability in plants usually leads to the reduction of water in the root-stem-leaf continuum (Barros *et al*., 1999; Catalina *et al*., 2010; Wang *et al*., 2018). Water deficit leads to the reduction of photolysis reaction in the photosystem that minimizes the formation of free hydrogen ions and electrons during electron transport chain systems, that in turn inhibits adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) production, that will be utilized in the dark reaction of photosynthesis (Nikolaeva *et al*., 2010;

Zargar *et al*., 2017). In addition, drought stress-induced abscisic acid (ABA) promotes stomatal closure to conserve the remaining internal water from loss and consequently lowering the internal CO_2 concentrations in the mesophyll and decreasing CO_2 fixation by inhibiting the synthesis of ribulose bisphosphate (RUBP) (Chaves *et al*., 2009; Zhang *et al*., 2021).

In this study, stomatal conductance also was strongly affected by the changes in the leaf water potential. As the drought-stress intensified, the stomatal conductance in the sensitive genotypes of *Ca*754 (Gs reduced by 70.24%, than well-watered) and *Ca*J-19 (63.85%, than well-watered) decreased more than in the relatively tolerant genotypes of *Ca*74112 (42.45%, than well-watered) and *Ca*74110 (54.68%, than well-watered). The decrease in stomatal conductance with decreasing leaf water potential was more substantial in *Ca*754 and *Ca*J-19 than in *Ca*74112 and *Ca*74110, indicating that the sensitivity of stomata to low leaf water potential conditions was higher. Similarly, the result of this study also identified the reduction of E under drought-stressed conditions. The relatively tolerant genotypes of *Ca*74112 and *Ca*74110 showed a reduction of E by 63.6% and 72.42%, respectively, whereas higher reductions were recorded in the sensitive genotypes of *Ca*J-19 and *Ca*754 where the reduction was 84.68% and 88.39%, respectively.

The decline in respiration in response to drought-stress is strongly correlated with the availability of water in the vascular system and the status of stomatal closure. The reduction of stomatal conductance under drought-stress is due to the production of abscisic acid (ABA), which pumps out the potassium to close the stomata to minimize transpiration loss (DaMatta *et al*., 2003; Nikolaeva *et al*., 2010). However, this stomata closure also inhibits the diffusion of CO_2 into the leaf, thus reducing net photosynthesis (A) (DaMatta *et al*., 2001; Verma *et al*., 2020). Similar to our findings, Gollan *et al*. (1985) and Almeida and Maestri (1997) reported that

stomata in sunflower and wheat started to close as the soil water content decreased even though the leaf water status was maintained at a high level. There is substantial evidence that drying of a small portion of the root system caused stomatal closure with negligible changes in the leaf water potential and that the phytohormone abscisic acid (ABA) may act as a root-sourced chemical messenger involved in mediating plant responses to soil drying (Xu and Zhou, 2008). ABA modulates stomatal aperture in leaves, although a difference between species in the water status for a rise in ABA has been identified. Beardsell and Cohen (1975) reported that drought-sensitive maize plants accumulated more ABA than tolerant sorghum plants in response to a decrease in leaf water potential, and that stomatal closure also was initiated at a higher water potential in maize than in sorghum. The difference among the genotypes in stomatal sensitivity at a high water potential may be affected by the difference in ABA accumulation. Similar to our findings, Dias *et al*. (2007), DaMatta *et al*. (2008), and da Silva *et al*. (2022), reported that relatively tolerant coffee genotypes, like *Ca*74110 and *Ca*74112, have the potential to display much-improved photosynthesis assimilation rate, stomatal conductance, and transpiration rate, unlike the sensitive genotypes, even under drought stress conditions. Dias *et al*. (2007) reported that, the positive correlation of water use efficiency with relative growth rate suggests that selection for high water use efficiency might increase growth and overall productivity.

3.4.4. Impact of drought stress on photosynthetic pigments concentration

Chlorophyll is an integral part of the leaf's ability to synthesize CO_2 and H_2O as products and oxygen with the help of sunlight in photosynthetic metabolism (Pirzad *et al*., 2011). In the present study, at the beginning of the stress experiment, *chl*-a, *chl*-b, and total chlorophyll contents showed no significant difference among the genotypes, but as the drought-stress intensifies it is found to cause pronounced reductions and was strongly correlated with the

reduction of relative water content and water potential. The relatively tolerant genotypes of *Ca*74112 and *Ca*74110 showed less reduction in chlorophyll-a, chlorophyll-b, and total chlorophyll contents than the sensitive genotypes of *Ca*J-19 and *Ca*754.

These reductions might be attributed to reduced water supply and a decrease in leaf water content, which restricts the movement of nutrients responsible for the synthesis of pigments, which ultimately declined the synthesis of photosynthetic pigments (Barros *et al*., 1999; Herbinger *et al*., 2002). Drought stress also influences the structure and functions of photosynthetic pigments by damaging the complex protein structures of the thylakoid membranes and decreasing the activities of one of the most abundant Calvin cycle RUBISCO enzymes (Taiz and Zeiger, 2010). The reduction is associated with the damage that occurred in the pigments of the light-harvesting complex proteins which directly impacts the photon absorption and electron transport chain (Catalina *et al*., 2010; da Silva *et al*., 2022; Maxiselly *et al*., 2022).

Jaleel *et al*. (2009) also reported that intensified drought-stress caused the degradation of photosynthetic pigments, damage to the membrane system, and the reduction of synthetase activity. Kirnak *et al*. (2001) have associated the reduction of chlorophyll concentrations with increased electrolyte leakage due to softening and breakage of the cell wall. Studies reported by Manivannan *et al*. (2007), Nikolaeva *et al*. (2010), and Mafakheri *et al*. (2010), indicated that with increased drought stress, leaves chlorophyll content showed a rapid decline in many crops, such as wheat, chickpea, and sunflower. Compared with the drought-sensitive *Ca*754 and *Ca*J-19 genotypes, the relatively drought-tolerant *Ca*74110 and *Ca*74112 genotypes exhibited more physiological protection mechanisms to reduce the influence of chlorophyll degrading enzyme, chlorophyllase.

3.4.5. Impact of drought stress on stomatal densities

The leaf stoma is a pivotal gate controlling the exchange of CO_2 and water vapor, although it is influenced by water status (Bertolino *et al*., 2019). Based on the works of Xu and Zhou (2008), the responses of stomatal density towards ws were an increase in stomatal density and a decrease in stomatal size. Similarly, the current study also found out that all the genotypes under well-watered conditions showed various stomatal densities, which are related to the genetic difference between them, and under exposure to drought-stressed conditions; the genotype's stomatal density was increased. The highest and lowest stomatal densities were recorded in the relatively tolerant genotype *Ca*74112 (275±3.19 mm^{-2}, tolerance response) and sensitive genotype *Ca*754 (218.33±1.67 mm^{-2}, sensitivity responses), respectively. The result also showed that stomatal density was positively correlated with A (r= 0.964), Gs (r= 0.991), and E (r= 0.990) and negative correlation with specific leaf area (r= -0.804). In the current study, drought-stress significantly increased stomatal density in the four genotypes, which is consistent with numerous previous studies, but contradictory to some results.

Previous studies on the responses of stomatal density towards drought stress reported various results and stomatal density increased in olive and solanum but decreased in ginger (Bosabalidis and Kofidis, 2002; Fu *et al*., 2013). In the meantime, Tanaka *et al*. (2013) stated that an increased stomatal density leads to an increased CO2 gas exchange and the photosynthesis rate. However, Xu and Zhou (2008) reported that under drought-stressed conditions, an increased stomatal density was associated with reduced net assimilation rate, stomatal conductance, and transpiration rate, which is similar to the finding of this study.

3.4.6. The magnitude of cell membrane stability and relative cell injury differ among genotypes under drought stress

Since the amount of electrolyte leakage is a function of membrane permeability, the membrane drought stability (the degree of damage resulting from water deficit) can be assessed in terms of electrolytic conductance (Rehman *et al*., 2016). Moreover, cell membrane stability and relative cell injury in response to drought-stress were considered to be an important measure of tolerance and sensitivity to be used to identify, select, and screen for genetic variation in drought tolerance among species and genotypes (Kumar *et al*., 2012). Therefore, this technique is considered to be useful in investigating membrane competence as affected by drought stress conditions.

The result of this study revealed that, at the end of the experiment, highly correlated and significant effect of drought-stress (r= 0.981, p<0.05) on cell membrane stability in the four genotypes under investigation. The relatively tolerant genotype *Ca*74112 showed a strong cell membrane stability of 82.5%, which is followed by *Ca*74110 with cell membrane stability of 73.31%, indicating a high level of drought-stress tolerance response. However, the least stability was displayed by the sensitive genotypes of *Ca*J-19 and *Ca*754 with cell membrane stability of 59.03% and 49.94%, respectively, indicating relative sensitivity towards drought stress. In the meantime, the relationship between cell membrane stability and relative cell injury is inversely proportional and weakly correlated (r= -1.00) where the highest and lowest relative cell injury was displayed by the sensitive genotype *Ca*754 (50.06%, sensitivity response) and the relatively tolerant genotype *Ca*74112 (17.5%, tolerance response), respectively.

Crops with higher cell membrane stability were also drought-stress tolerant, and the stability of cell membranes under drought-stress was linked to drought-induced chaperon production,

proline content, and phospholipids saturation levels (Quilambo, 2004; ElBasyoni *et al*., 2017). In response to drought-stress, the level of membrane stabilizers, i.e., heat-shock proteins, and saturated lipids, increased in drought-stress tolerant genotypes to maintain the membrane permeability and integrity (Suzuki *et al*., 2014; Pandey *et al*., 2015). Prasch and Sonnewald (2013) and Zhou *et al*. (2017), also reported that photosynthetic products, such as sugars, explicitly accumulated during drought-stressed conditions. Furthermore, the current study also suggests further investigation into the relationship between cell membrane stability and membrane stabilizing factors (proline, phospholipids, and glycolipids) to better understand the drought-stress tolerance mechanism in *C. arabica* genotypes.

3.4.7. Multivariate analysis of parameters analysed in this study

The overall result showed that, under drought-stressed conditions, the main differences among the *C. arabica* genotypes were recorded in the case of vegetative growth, gas exchange, water parameters, stomatal densities, and cell membrane stabilities. The changes in the growth and physiological parameters of the coffee genotypes varied under drought-stress, and the responses were different in different periods, which indicated that genotypes have different ways to adapt to drought stress. The sensitivity to drought stress is different, so the response time is also different, and the genotypes may be in different response stages simultaneously (DaMatta *et al*., 2007). During continuous drought stress, plants experience stress, adaptation, injury, and repair. The comprehensive adjustment of different response mechanisms in different stress stages constitutes the overall drought resistance of plants (Tounekti *et al*., 2018). According to de Oliveira *et al*. (2022), plants' have the same tolerant and sensitivity response capacities, belong to the same PCA group category, and respond similarly in terms of, as in this study, vegetative and physiological performance.

Similarly, in this study, the responses of the relatively tolerant genotypes of *Ca*74112 and *Ca*74110, and sensitive genotypes of *Ca*J-19 and *Ca*754, responded in a closely related manner towards drought stress conditions, where the former responded with superior vegetative and physiological responses having a tolerant capacity than the latter.

3.5. Conclusions

Drought stress hampered the morphological and physiological performances of *C. arabica* genotypes, nevertheless, prolonged drought stress was found to be severe for all the studied traits. Along with the intensification of drought stress, significant reduction in terms of shoot and root growth, gaseous exchange, pigment contents, and cell membrane stability were also recorded, compared to the well-watered conditions. Besides, variations in response to genotypes were apparent against drought stress, and the relatively tolerant genotypes of *Ca*74112 and *Ca*74110 performed comparatively better than the sensitive genotypes of *Ca*754 and *Ca*J-19 who responded poorly. Drought tolerance PCA ranking showed a high relationship between the drought-stressed conditions and tolerance behavior of the relatively tolerant (*Ca*74112 and *Ca*74110) genotypes. Based on these findings, in order to delve further into the mechanisms of drought stress tolerance in arabica coffee genotypes, it is imperative to explore the role of membrane stabilizing factors, metabolites, and proteins (aquaporins) related to water transport and gene expression studies.

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4. CHAPTER FOUR

Drought Stress Responses in Arabica Coffee (*Coffea arabica* L.) Genotypes: Physiological and Metabolic Insights

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Abstract

Understanding the impact of drought stress on Arabica coffee physiology and metabolism is essential in the pursuit of developing drought-resistant varieties. In this study, we explored the physiological and metabolite changes in coffee genotypes exhibiting varying degrees of tolerance to drought—namely, the relatively tolerant *Ca*74110 and *Ca*74112, and the sensitive *Ca*754 and *Ca*J-19—under well-watered conditions and during terminal drought stress periods over two-time intervals (0 and 60 days following the onset of stress). The Metabolites profiling conducted uncovered significant associations between growth and the physiological characteristics of coffee genotypes with distinct drought tolerance behaviors. Initially, no marked differences were observed among genotypes or treatments. However, at the 60-day post-drought onset, notably higher shoot growth, biomass, CO_2 assimilation, pigments, and various physiological parameters were evident, particularly in the relatively tolerant genotypes. Metabolite profiling revealed elevations in glucose, maltose, amino acids, organic acids, and decreases in other metabolites, more pronounced in drought-tolerant genotypes. These alterations were more pronounced in the drought-tolerant genotypes, indicating a correlation with enhanced compatible solutes and energy-associated metabolites crucial for drought tolerance mechanisms. This research introduces GC-MS based metabolome profiling to Ethiopian coffee, shedding light on the intricate responses to drought stress and paving the way for the potential development of drought-resistant coffee seedlings in intensified agro-ecological zones.

Keywords: Drought stress, *Coffea arabica*, growth, gas exchanges, metabolites, network analysis

4.1. Introduction

Coffee stands as a vital global agricultural commodity, trailing only oil in importance. Its production in tropical and subtropical regions sustains millions of livelihoods (Taye Kufa, 2012; Rodrigues *et al*., 2021). *Coffea arabica* L. accounts for over 70% of the world's coffee and is famed for its excellence (Elmar and Jean-Francois, 2006). Brazil leading Arabica coffee production at 44%, with Ethiopia contributing 5% (FAOSTAT, 2021). Ethiopia, a top Arabica coffee producer in Africa, exports about 471,000 tons yearly, yielding 0.71 tons per hectare (CSA, 2021; ECX, 2021; Habtamu Chekol *et al*., 2023). The looming specter of global climate change, however, threatens *C. arabica* cultivation, with water scarcity and drought. This poses significant challenges to coffee cultivation, disrupting suitable regions, yield, and quality, and inviting pests and diseases, causing economic losses (Leon-Burgos *et al*., 2022; da Silva *et al*., 2022).

Plants instinctively adjust catabolic and anabolic systems during drought, altering metabolic pathways to protect against damage (Arbona *et al*., 2013; Kumar *et al*., 2021). This adaptation's mechanism hinges on species, genotype, and stress intensity (Witt *et al*., 2012; Hochberg *et al*., 2013). Metabolic adjustment involves accumulating compatible solutes, influencing pathways like sugar synthesis, photosynthesis, and more (Fiehn *et al*., 2000). Certain metabolites increase during drought stress, like proline, serine, valine, and betaine, fostering tolerance (Guo *et al*.,2018; Lozano-Elena *et al*., 2022), while others decrease, like Myo-inositol and glutamate (Rabara *et al*., 2017). Metabolite accumulation helps cell turgor maintenance, osmotic potential reduction, and oxidative damage protection (Drapal *et al*., 2020). Stress-resilient plants often sustain higher stress-related metabolite levels even under normal conditions (Hochberg *et al*., 2013; Obata *et al*., 2015). Despite the importance of metabolomic components in drought

tolerance, insights into *C. arabica*'s metabolomics under drought remain limited (Fabregas and Fernie, 2019). Understanding drought stress adaptation necessitates studying metabolomic responses in drought-tolerant and sensitive genotypes (Impa *et al*., 2019).

Metabolomic analysis, utilizing techniques like gas/liquid chromatography–mass spectrometry and nuclear magnetic resonance, reveals the level of small-molecule metabolites precisely (Roessner and Beckles, 2009; Schwender, 2009; Lu *et al*., 2017). Nevertheless, understanding coffee's intricate genetic and molecular aspects remains a significant hurdle (Rodrigues *et al*., 2021). Recent focus on coffee has illuminated metabolite responses to high temperature (Obata *et al*., 2015), water logging (Caine *et al*., 2019), elevated CO_2 (Impa *et al*., 2019), and drought (Rodrigues *et al*., 2021). However, previous research in Ethiopia has predominantly approached drought stress in coffee from an agronomic and yield perspective, with limited comprehensive metabolomic profiling. Given metabolites' role in osmoregulation during drought stress, bolstering coffee drought research with metabolomic investigations is crucial. Thus, this study aims to characterize and profile metabolites' response to drought in tolerant and sensitive *C. arabica* genotypes, enhancing our grasp of drought adaptation and possibly guiding robust coffee varieties. By understanding these mechanisms, we may pave the way for more climate-resilient coffee varieties.

4.2. Material and methods

4.2.1. Plant material

The study used four *C. arabica* L. (*Ca*) genotypes sourced from Jimma Agricultural Research Center (JARC). The selection of these genotypes was based on their drought tolerance, comprising both relatively tolerant (*Ca*74112 and *Ca*74110) and sensitive (*Ca*754 and *Ca*J-19) genotypes, as previously reported by Tesfaye Shimber (2018[b]) and Habtamu Chekol *et al*.

(2023). Our experimental focus was to investigate the interplay between growth, physiological performance, and metabolite responses within adult coffee genotypes. Adhering to the guidelines from WCR (2021), we transplanted germinated coffee plants once they exhibited the first leaf pair, and were disease-free, had 3-5 cm tall stems (hypocotyls), and 2-3 cm secondary roots. These germinants were transplanted in 5 L plastic pots, with the side of the pot covered with aluminum foil to prevent excessive heat buildup. The pots, with drainage holes, were filled with 4 L of mixed topsoil, compost, and sand (2:1:1 ratio, pH 5.4-6.8). To address specific nutritional requirements and align with distinct coffee growth stages, we added 2.0 g of NPK/DAP fertilizers 5-7 cm below the seedlings. Subsequently, uniform-looking seedlings of each genotype were placed within a greenhouse environment and received consistent watering prior to the initiation of the drought stress treatments.

4.2.2. Growth condition and experiment design

The research was conducted within a controlled greenhouse condition where the relative humidity ranges between 50-70%, with an average temperature of 24.5°C and a photon flux density of 850 ± 13 μmol $m^{-2}s^{-1}$. Coffee genotypes (240 days aged), free from disease or nutrient deficiencies, were used for the study, and subjected to two conditions: well-watered (*ww*) and drought-stressed (*ws*). Under *ww*, soil moisture of 60–80% field water capacity, seedlings were irrigated every 3-4 days. In contrast, for the *ws* conditions, seedlings were initially fully irrigated to the same field water capacity and subsequently subjected to drought conditions by withholding water until the experiment's end (around 300 days of coffee age). The study used a completely randomized block design (CRBD) with four genotypes and two water regimes, each replicated ten times, totaling 80 coffee plants.

To evaluate the coffee growth and physiological performance in response to drought stress, at 10-day intervals until the end of the experiment (for around 60 days), shoot height, leaf relative water content, stomatal conductance, and net carbon assimilation rate were measured. Besides, evaluations of pigments of the coffee genotypes were conducted at the beginning of the experiment (0 timelines) and end of the experiment (60 days after drought started).

For the metabolite analysis, we sampled the third matured leaf from upper new flesh growth at two distinct time points during the study: at the start of the experiment (0 days) and end of the experiment (after 60 days of the drought implementation period). These fresh biomass leaf samples were snap frozen in liquid nitrogen and stored at -80°C for further metabolite analysis.

4.2.3. Leaf relative water content

At 10-day intervals until the end of the experiment (for around 60 days), relative water content (RWC) from representative leaves of the coffee genotypes was calculated based on the following formula of Barrs and Weatherley (1962):

$$\mathrm{RWC} = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100 \quad (1)$$

where FW represents the leaf fresh weight, DW represents the leaf dry weight, and TW represents the leaf turgid weight.

Leaves fresh weight was measured, and samples were soaked in distilled water for 2 hours (h) at room temperature (20–22°C) and turgid weight was determined. Furthermore, the samples were dried to a constant weight at 70°C, and dry weight was determined. Sample weights were measured using balance to an accuracy of 0.0001 g (Sartorius, Bangalore, India).

4.2.4. Gas exchange measurements

At 10 day intervals until the end of the experiment (for around 60 days), instantaneous gas exchange measurements were measured periodically. The rate of stomatal conductance (Gs, mol

H_2O $m^{-2}s^{-1}$) and net carbon assimilation (A_{net}, µmol CO_2 $m^{-2}s^{-1}$) were collected using an open gas exchange system LI-6400 (LI-COR, Lincoln, Nebraska, USA) adjusted at 400 µmol CO_2 mol^{-1} air reference CO_2 concentration, 1000 µmol $m^{-2}s^{-1}$ photosynthetic photon flux density, and 500 µmol s^{-1} flow rates. The measurements were conducted on a young and fully expanded leaf, between 9:00 and 11:00 a.m.

4.2.5. Content of photosynthetic pigments

For pigments analysis (chlorophylls), at 0 and 60 days after the start of drought treatment, leaf discs were collected from healthy and fully expanded leaves which were used for gas exchange measurements, and the concentration of pigments were analyzed using a UV-VIS spectrophotometer (Model 3092, Maharashtra, India). The concentration of chlorophyll a (*chl*-a), and chlorophyll b (*chl*-b), were measured based on the following formulas (Lichtenthaler, 1987):

$$\textit{Chl-a}\ (\text{mg/g tissue}) = 12.25\ A_{663.2} - 2.79\ A_{646.8} \quad (2)$$

$$\textit{Chl-b}\ (\text{mg/g tissue}) = 21.50\ A_{646.8} - 5.10\ A_{663.2} \quad (3)$$

where *Chl*-a represents the content of chlorophyll-a (mg g^{-1} tissue), and *Chl*-b represents the content chlorophyll-b (mg g^{-1} tissue).

4.2.6. Vegetative growth measurements

At 10 day intervals until the end of the experiment (for around 60 days), in response to drought stress, the stem height (SH, cm, using meter scale) growth performance were measured. Following sample harvesting (plants at around 300 days of age), shoot fresh biomass (g) and dry biomass (g, oven-dry biomass at 70°C for 24 h) were measured using sensitive balance (0.0001 g accuracy, Sartorius, Bangalore, India).

4.2.7. Metabolite analysis

To analyze metabolites, the dried leaf biomass samples, frozen using liquid nitrogen and stored at -80°C, of the well-watered and drought stressed conditions from 0TL and 60TL, were ground into constant weight of fine powder under liquid nitrogen using Mortar and pestles. The powder was oven-dried to a constant weight at 70°C for a period of 24 h. Approximately 100 mg powder was weighed, and were extracted in a 1 ml methanol:chloroform:water extraction solution (2.5:1:1 v/v) (Lisec *et al*., 2006). The mixture was thoroughly vortexed (MX-S, Scilogex, Rocky Hill, USA) and kept in an orbital shaker (OS- 20Pro, Joan Lab Equipment Co., Ltd., Huzhou, China) for duration of 15 minutes. Following this initial preparation, the samples underwent centrifugation (MSLZL19, Neuvar, Palo Alto, CA, USA) for 10 minutes at 12000 revolutions per minute (rpm) and placed at 4°C. The resulting supernatant was then carefully transferred to 2 mL screw-top tubes, mixed with 300 µl of chloroform and 300 µl of Mass Spec (MS) grade water and then centrifuged at 20,000 × g for 2 min. Subsequently, 100 µl of the polar phase (water-methanol phase) was dried in a vacuum concentrator (Vacufuge Plus, Eppendorf, Hamburg, Germany) at 30°C for a period of 3 hours, and stored at -80°C.

The dried polar extracts were derivatized with 40 µL of 20 mg mL^{-1}methoxyamine hydrochloride, followed by 70 µL of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (TMS derivatization) and 20 µL mL^{-1} of a mixture of fatty acid methyl esters (FAMES). For metabolite analysis, the study utilized Gas Chromatography-Mass Spectrometry (GC-MS), employing an Agilent 7890 system coupled with a DB-5MS capillary column coated with a 5% diphenyl and 95% dimethylpolysiloxane mixture. During injection, an aliquot of the analyte (1 µL) was injected in a splitless mode. Helium served as the carrier gas, with a specific temperature program ranging from 90°C to 285°C. Peaks were manually annotated, and ion intensity was

determined and metabolites were identified through systematic comparison with established reference library derived from the Golm Metabolome Database (Kopka *et al*., 2005), based on retention time and indices, and mass spectrum, that enabling us to gain insights into the intricate metabolic profiles of the coffee genotypes under study. In order to understand the alteration of the metabolites, the resulting ion intensity of relative concentration were transformed and normalized for the removal of measurement bias, and set for further statistical analysis.

4.2.8. Statistical analysis

Statistical analyses of the collected data were performed using Analysis of Variance test in SigmaPlot version 13 (Systat Software Inc., San Jose, CA, US). To identify significant differences among the experimental groups, post hoc multiple comparisons were performed using Tukey's honest significant difference test ($p<0.05$). The dataset was transformed in Past version 4.0.3 (Hammer *et al*., 2001). Pearson correlation analysis, between all metabolite pairs and among metabolite, growth, and physiological traits, was performed after checking the assumptions of normality using the Shapiro–Wilk test. To reconstruct a metabolite network that would capture the coordinated changes in the metabolic profiles, threshold values were determined. Network visualization of metabolites was performed using Cytoscape version 3.10.1 (Shannon *et al*., 2003), and the number of edges, number of nodes, edge to node ratio, network density, average node degree, characteristics path length, clustering coefficient, network heterogeneity, network diameter, network radius, and network centralization were investigated. To construct correlation-based networks of significant correlations, $r>0.8$ threshold values were applied. Principal component analyses were performed on the transformed (*ws*/*ww*), using RStudio (version 4.2.1).

4.3. Results

4.3.1. Shoot growth and biomass were affected by drought stress treatments

Distinct variations in growth were evident among the four coffee genotypes following a 60-day period of drought-induced stress. When compared to well-watered coffee genotypes, those exposed to drought stress exhibited notably lower shoot height, shoot fresh and dry biomass at the end of the stress period, displaying statistically significant differences ($p<0.05$). Within the conditions of drought stress, markedly higher stem height (see Figure 4.1), shoot fresh biomass (see Figure 4.2A), and shoot dry biomass (see Figure 4.2B) were observed in the relatively tolerant genotypes *Ca*74112 (18.16±2.42 cm, 9.3 g, and 2.71 g) and *Ca*74110 (15.35±1.19 cm, 7.11 g, and 2.01 g), whereas lower values were recorded in the sensitive genotypes of *Ca*754 (11.4±1.3 cm, 4.43 g, and 1.17 g) and *Ca*J-19 (13.25±1.19 cm, 5.73 g, and 1.57 g), respectively. In comparison to the well-watered genotypes, at the conclusion of the experiment under drought stress conditions, the relatively tolerant genotypes *Ca*74112 (38.33%) and *Ca*74110 (43.59%) displayed the least reduction in shoot growth, while the sensitive genotypes *Ca*754 (50.84%) and *Ca*J-19 (47.17%) experienced higher reductions in shoot heights. Similarly, at the end of the experiment, the least reduction in both shoot fresh and dry weight was observed in the relatively tolerant genotypes *Ca*74112 (31.01%, 30.87%) and *Ca*74110 (41.34%, 41.23%), compared to the sensitive genotypes *Ca*754 (62.14%, 62.13%) and *Ca*J-19 (60.21%, 60.25%).

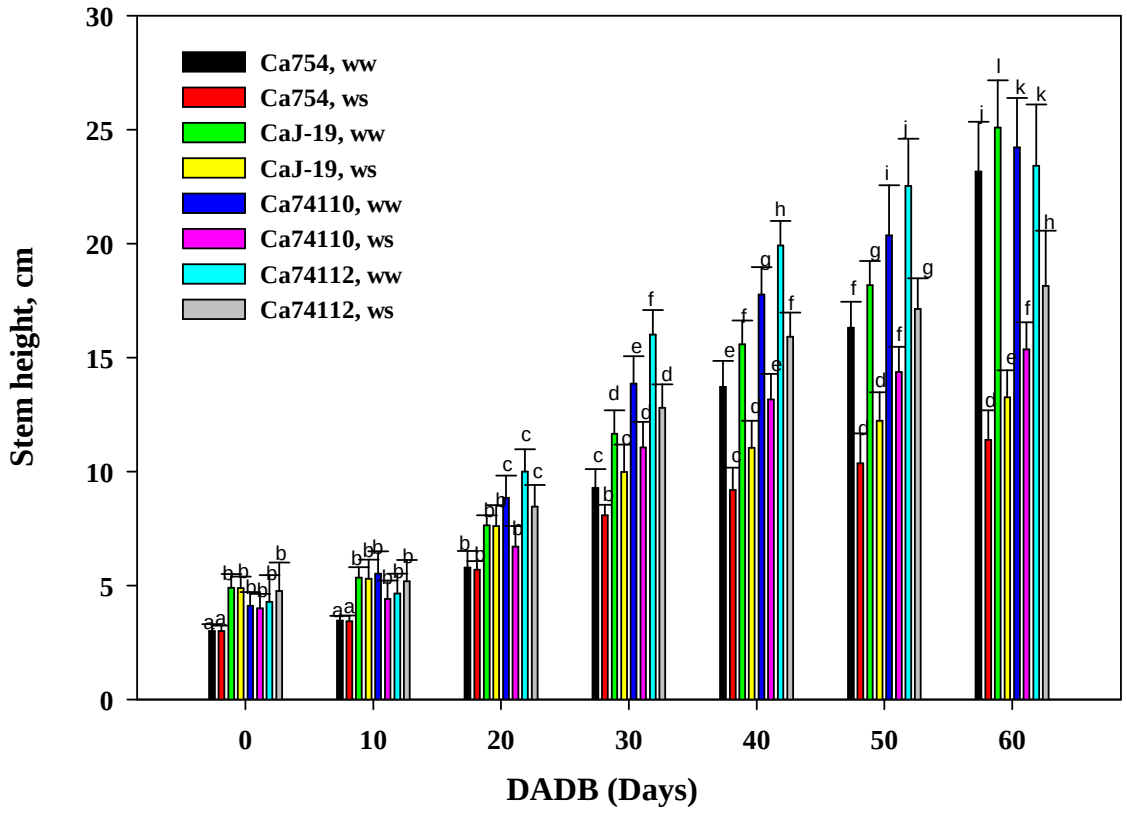

Figure 4.1. Effects of drought stress on stem length of *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112 coffee genotypes, under drought stress (*ws*) and well water (*ww*) conditions, at different DADB (days after drought stress begins). Bars (means±SD, *n*=10 replicates per genotype) with the same letter indicate no significant difference.

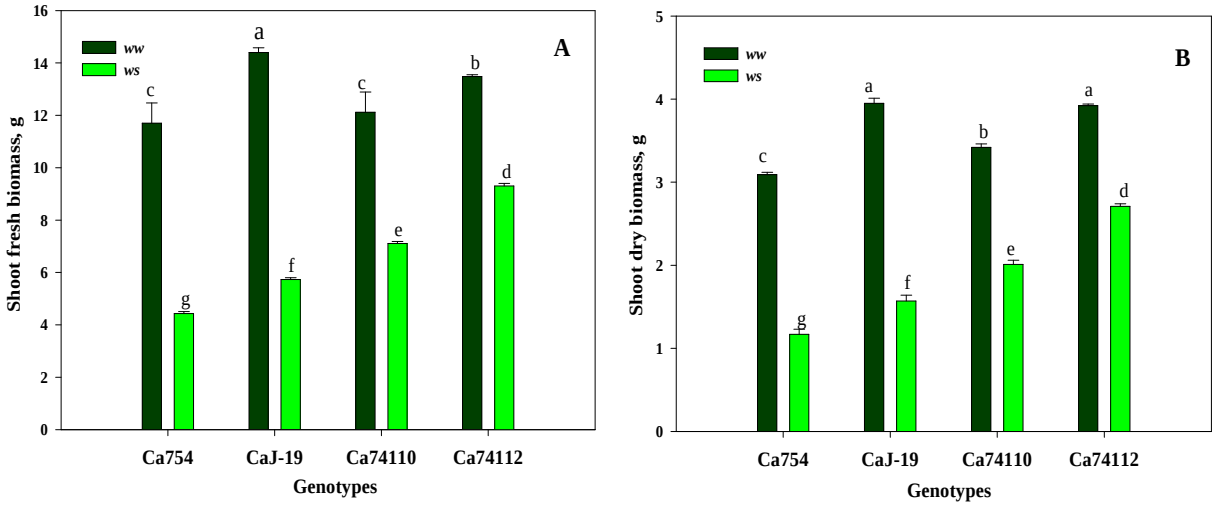

Figure 4.2. Effects of drought stress on shoot (**A**) fresh biomass, and (**B**) dry biomass of *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112 coffee genotypes, under drought stress (*ws*) and well water (*ww*) conditions, 60 days after drought stress begins. Bars (means±SD, *n*=10 replicates per genotype) with the same letter indicate no significant difference.

4.3.2. Difference of relative water content of coffee genotypes under drought stress

In well-watered conditions, there were no significant ($p>0.05$) differences among the genotypes where the relative water content value ranges between 81.16%-82.76%. However, under drought-stressed conditions, at the end of the experiment, the mean relative water content was significantly ($p<0.05$) lower than those in well watered conditions and the value of RWC was different among coffee genotypes, and a higher RWC were identified in the relatively tolerant genotypes of *Ca*74112 (48.11±0.8%, with 41.89% reduction than well-watered (*ww*) conditions) and *Ca*74110 (43.40±0.29%, with 46.74% reduction than well-watered (*ww*) conditions), and a lower RWC were recorded in the sensitive genotypes of *Ca*754 (30.24±0.21%, with 62.74% reduction than *ww* conditions) and *Ca*J-19 (32.57±0.13%, with 60.32% reduction than *ww* conditions) (see Figure 4.3A).

4.3.3. Influence of drought stress in stomatal conductance among the coffee genotypes

At the initial stage, no significant differences in stomatal conductance (Gs, mmol $m^{-2}s^{-1}$) were observed between plants grown under drought stress and those under well-watered conditions. However, by the end of the experiments, noticeable variations in Gs were evident across genotypes. Among the drought-stressed plants, the highest Gs was recorded in the relatively tolerant genotypes *Ca*74112 (60.27 ± 1.39 mmol $m^{-2}s^{-1}$) and *Ca*74110 (46.57 ± 0.9 mmol $m^{-2}s^{-1}$), while the minimum Gs was recorded in the sensitive genotypes *Ca*J-19 (36.86 ± 0.72 mmol $m^{-2}s^{-1}$) and *Ca*754 (30.3 ± 0.87 mmol $m^{-2}s^{-1}$). Because of the imposed drought stress, there were reductions in Gs by 38.32%, 53.31%, 63.85%, and 69.34% in the genotypes *Ca*74112, *Ca*74110, *Ca*J-19, and *Ca*754, respectively (see Figure 4.3B).

4.3.4. Drought stress associated variation in carbon assimilation among coffee genotypes

The impact of drought stress on net carbon assimilation rate (A_{net}, µmol CO_2 $m^{-2}s^{-1}$), stomatal conductance (Gs, mmol $m^{-2}s^{-1}$) and transpiration rate (E, mmol $m^{-2}s^{-1}$) were examined. There were no significant differences in the CO_2 assimilation rate among the genotypes grown under control conditions. However, when subjected to drought stress conditions, all genotypes displayed notably distinct reductions in CO_2 assimilation rate, exhibiting a gradual decline throughout the experiment. The relatively tolerant genotypes of *Ca*74112 (2.91±0.12 µmol $m^{-2}s^{-1}$) and *Ca*74110 (2.31±0.07 µmol $m^{-2}s^{-1}$) recorded higher A_{net} values, while lower values were observed in the sensitive genotypes *Ca*J-19 (1.57±0.14 µmol $m^{-2}s^{-1}$) and *Ca*754 (1.02±0.1 µmol $m^{-2}s^{-1}$). In assessing the impact of drought stress on the reduction of CO_2 assimilation rate, the sensitive genotype *Ca*754 experienced the highest reduction rate (85.96%), significantly greater

than the lowest reduction observed in the tolerant genotype *Ca*74112 (59.85%) (see Figure 4.3C).

4.3.5. Variations of photosynthetic pigments under drought stress among coffee genotypes

Across all tested coffee genotypes, drought stress led to a notable decline in *Chl*-a (see Figure 4.4A) and *Chl*-b (see Figure 4.4B) content, while in well-watered plants, chlorophyll levels remained relatively stable throughout the experiment. By the end of the drought stress period, genotype *Ca*74112 recorded the significantly highest and lowest *Chl*-a and *Chl*-b values (1.09 mg g^{-1}fw and 0.21 mg g^{-1}fw, respectively), while *Ca*754 had the lowest values (0.63 mg g^{-1}fw for *Chl*-a and 0.14 mg g^{-1}fw for *Chl*-b) ($p<0.05$). Comparing the reduction rates of *Chl*-a and *Chl*-b due to drought stress, the sensitive genotype *Ca*754 displayed the highest reduction rates (56.96% for *Chl*-a and 57.14% for *Chl*-b), whereas the tolerant genotype *Ca*74112 exhibited lower reduction rates (28.56% for *Chl*-a and 38.72% for *Chl*-b).

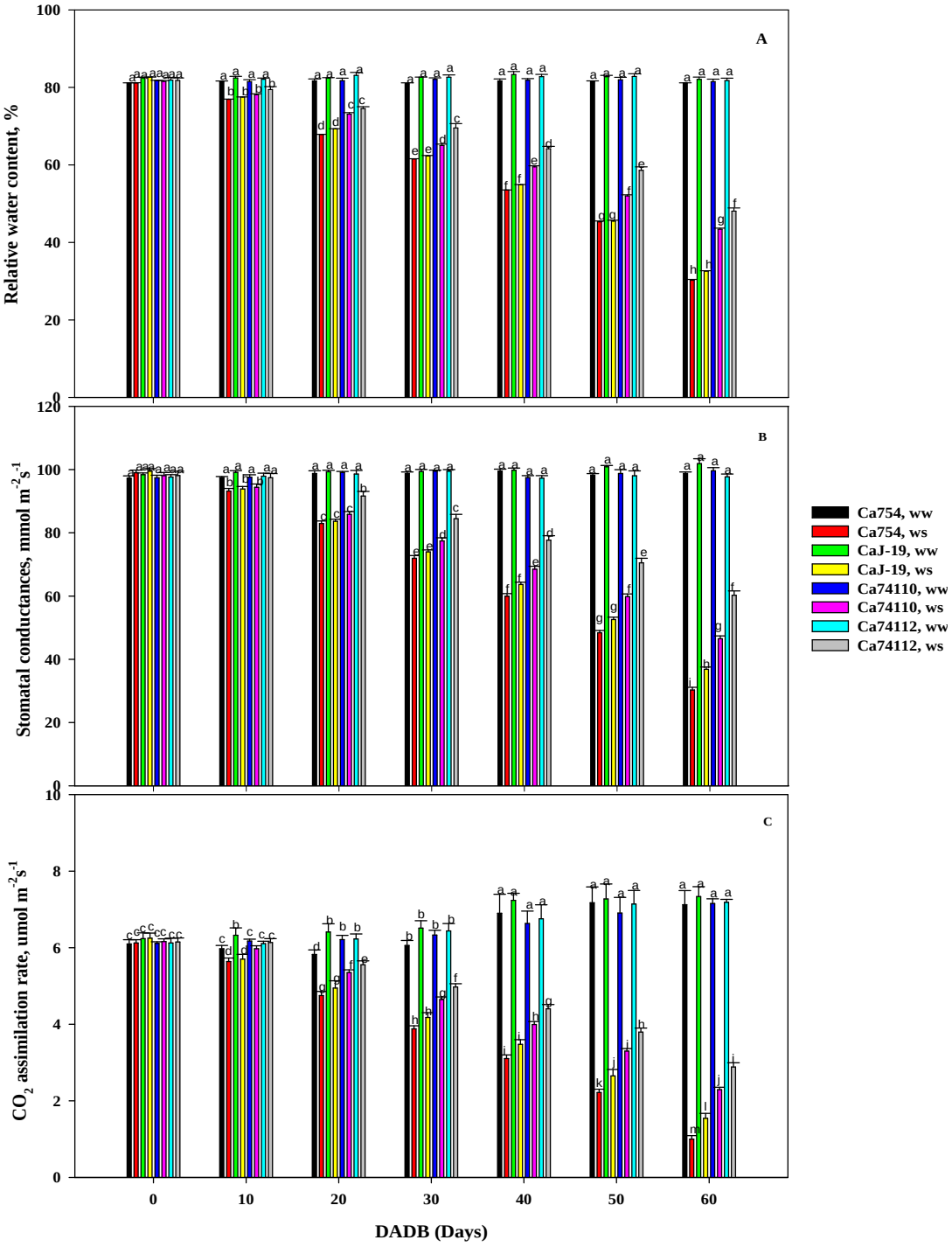

Figure 4.3. Effects of drought stress on (**A**) relative water content, (**B**) stomatal conductance, and (**C**) net CO_2 assimilation rate of *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112 coffee genotypes, under drought stress (*ws*) and well water (*ww*) conditions, at different DADB (days after drought stress begins). Bars (means±SD, *n*=10 replicates per genotype) with the same letter indicate no significant difference.

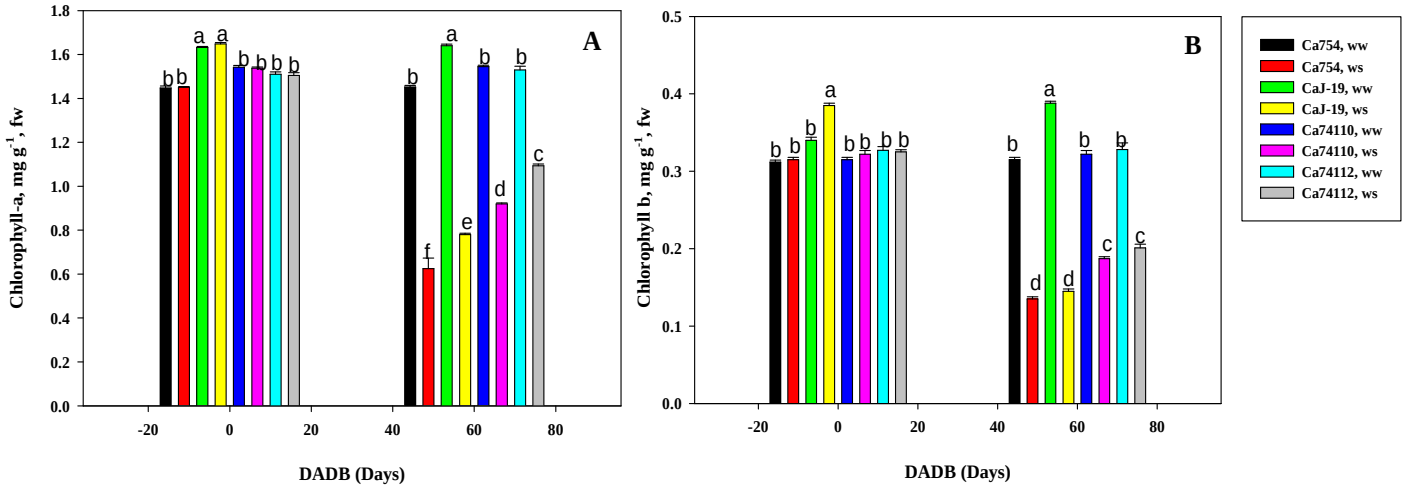

Figure 4.4. Effects of drought stress on (**A**) chlorophyll-a, and (**B**) chlorophyll-b of *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112 coffee genotypes, under drought stress (*ws*) and well water (*ww*) conditions, at 0 and 60 DADB (days after drought stress begins). Bars (means±SD, *n*=10 replicates per genotype) with the same letter indicate no significant difference.

4.3.6. Alteration of metabolites under drought stress conditions

To investigate the molecular changes associated with drought tolerance, we examined metabolic responses at two distinct time points: 0 and 60 days into the drought implementation periods. A profiling analysis revealed 50 identified metabolites spanning sugars, amino acids, and intermediates from pathways such as the tricarboxylic acid cycle (TCA), glycolysis, γ-aminobutyric acid (GABA) shunt, and shikimic pathways. The relative concentrations of each metabolite are represented as logarithmic (Log_2) transformed fold changes (*ws*/*ww*) (see Figure 4.5).

Initially, no significant differences in metabolite alterations were observed between relatively tolerant and sensitive coffee genotypes in both treatment groups. However, after 60 days of drought stress initiation (DADB), a noteworthy shift occurred. The relatively tolerant genotypes, *Ca*74112 (with 46 altered metabolites - 30 up and 16 down) and *Ca*74110 (with 46 altered

metabolites - 29 up and 17 down), displayed significantly higher alterations compared to the sensitive genotypes, *Ca*J-19 (42 altered metabolites - 23 up and 19 down) and *Ca*754 (40 altered metabolites - 22 up and 18 down). At the outset of the study (0TL), no significant metabolite alterations were evident between the well-watered/control (*ww*) and drought-stressed (*ws*) treatments. However, by 60 DADB, substantial alterations in metabolite accumulation were observed compared to the control ($p<0.01$).

At 60 DADB, the relatively tolerant genotypes exhibited markedly higher alterations and increased metabolite accumulation compared to the sensitive genotypes, with a notably greater magnitude. Specifically, in the relatively tolerant genotypes (*Ca*74110 and *Ca*74112), the most substantial up-accumulation was observed in glucose (402 and 528-fold), tryptophan (167 and 401-fold), maltose (122.4 and 95.6-fold), galactose (25.8 and 37.7-fold), L-Cysteine (2 and 48.44-fold), lactose (27.12 and 32.87-fold), lysine (14.18 and 22.71-fold), methionine (20.4 and 18.15-fold), leucine (9.66 and 14.45-fold), pyruvic acid (13.06 and 12.83-fold), ribose (8.82 and 9.59-fold), and other amino acids and organic acids. Conversely, in *Ca*754, *Ca*J-19, *Ca*74110 and *Ca*74112 respectively, significant reductions ($p<0.05$) were observed in glutamate (0.13, 0.16, 0.15 and 0.15-fold), aconitic acid (0.65, 0.56, 0.37 and 0.28-fold), glucose-6-phosphate (0.53, 0.68, 0.12 and 0.33-fold), γ-aminobutyric acid (0.49, 0.39, 0.36 and 0.48-fold), fructose-6-phosphate (0.74, 0.81, 0.29 and 0.41-fold), asparagines (0.38, 0.29, 0.51 and 0.57-fold), glutamine (0.23, 0.22, 0.19 and 0.59-fold), aspartate (0.66, 0.47, 0.56 and 0.63-fold), alanine (0.82, 0.98, 0.74 and 0.64-fold), myo-inositol (0.69, 0.68, 0.67 and 0.68-fold), and citric acid (0.55, 0.27, 0.63 and 0.86-fold) among the four genotypes (Appendix 12 and 20).

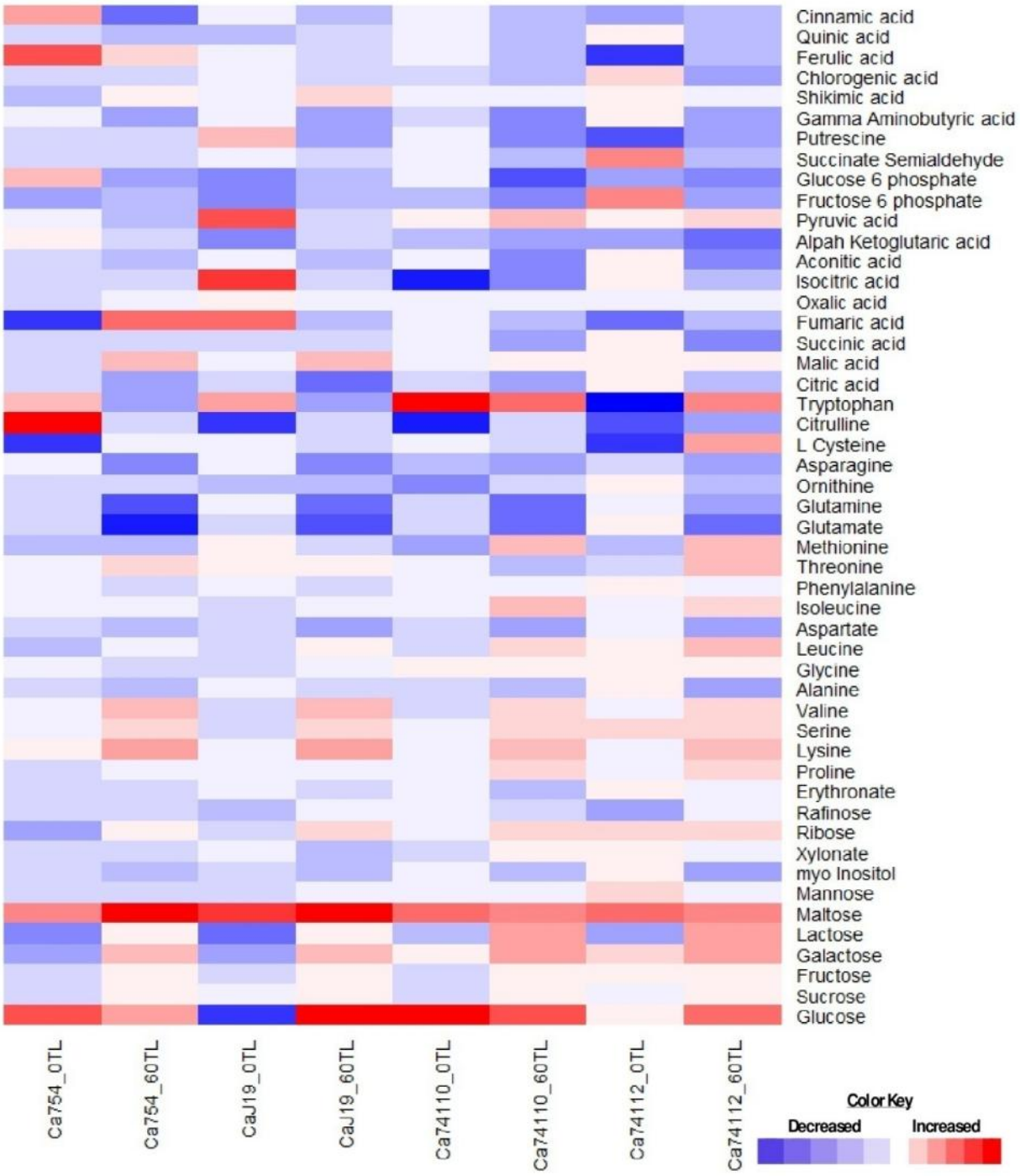

Figure 4.5. Metabolic responses to drought stress in leaves of *Ca*754, *CaJ*-19, *Ca*74110, and *Ca*74112 coffee genotypes. Each heatmap box represent logarithmic (Log_2) transformed fold changes (*ws*/*ww*, drought stressed/well-watered) of selected leaf metabolites at 0 and 60 days after drought stress begins. Red, Blue and white represent an increase, decrease, and in between, in terms of metabolite alteration. TL refers the experiment time line.

4.3.7. Multivariate analysis

4.3.7.1. Correlation of specific metabolites with growth and physiology

An interesting aspect of this study was to combine knowledge from growth and physiological response of coffee genotypes with metabolic data. For this purpose, a correlation analysis of the growth and physiological parameters with metabolic response, at 60 DADB, was performed. Among the highly increased sugar metabolites, a strong positive correlation of glucose, maltose, and galactose, with shoot fresh biomass (*r*= 0.89; *r*= 0.837; *r*= 0.927, respectively), RWC (*r*= 0.939; *r*= 0.942; *r*= 0.993, respectively), A_{net} (*r*= 0.946; *r*= 0.905; *r*= 0.964, respectively), and Gs (*r*= 0.884; *r*= 0.842; *r*= 0.936, respectively) were observed. Besides, a strong positive correlation of tryptophan, L-Cysteine, and proline with shoot fresh biomass (*r*= 0.867; *r*= 0.886; *r*= 0.908, respectively), RWC (*r*= 0.969; *r*= 0.771; *r*= 0.987, respectively), A_{net} (*r*= 0.917; *r*= 0.805; *r*= 0.948, respectively), and Gs (*r*= 0.88; *r*= 0.891; *r*= 0.918, respectively) were observed. From the TCA cycle and glycolysis pathway, a strong positive correlation for malic and pyruvic acid with RWC (*r*= 0.922; *r*= 0.968, respectively), A_{net} (*r*= 0.841; *r*= 0.923, respectively), and Gs (*r*= 0.808; *r*= 0.878, respectively) were observed. A strong negative correlation of myo-inositol with shoot fresh biomass (*r*= -0.613), RWC (*r*= -0.695), A_{net} (*r*= -0.713), Gs (*r*= -0.595), and E (*r*= -0.61) were observed. Glutamate was weakly correlated with shoot fresh biomass (*r*= 0.539), RWC (*r*= 0.426), A_{net} (*r*= 0.562), and Gs (*r*= 0.496). α-ketoglutaric acid and glucose-6-phosphate were negatively correlated with shoot fresh biomass (*r*= -0.966; *r*= -0.573, respectively), RWC (*r*= -0.933; *r*= -0.758, respectively), A_{net} (*r*= -0.933; *r*= -0.682, respectively), and Gs (*r*= -0.976; *r*= -0.584, respectively) (Appendix 13).

4.3.7.2. Network analysis of metabolic alteration

The 60 days of drought stress caused an increase in the number of edges, network densities, average node degree and other key values of metabolites network analysis, and the increased value was more characterized in the relatively tolerant than the sensitive coffee genotypes (see Figure 4.6 and 4.7). Thus, at $r > 0.8$, the mean values of network analysis in the relatively drought tolerant genotypes showed that, they had 87 nodes, 1250 number of edges, 14.4 to 1 edge to node ratio, 0.359 network density, 29.762 average node degree, 4.268 characteristics path length, 0.924 clustering coefficient, 0.385 network heterogeneity, 10 network diameter, 5 network radius, and 0.126 network centralization, while the sensitive genotypes had 80 nodes, 1411 number of edges, 17.6 to 1 edge to node ratio, 0.907 network density, 36.293 average node degree, 1.094 characteristics path length, 0.991 clustering coefficient, 0.211 network heterogeneity,3 network diameter, 2 network radius, and 0.071 network centralization (Appendix 14).

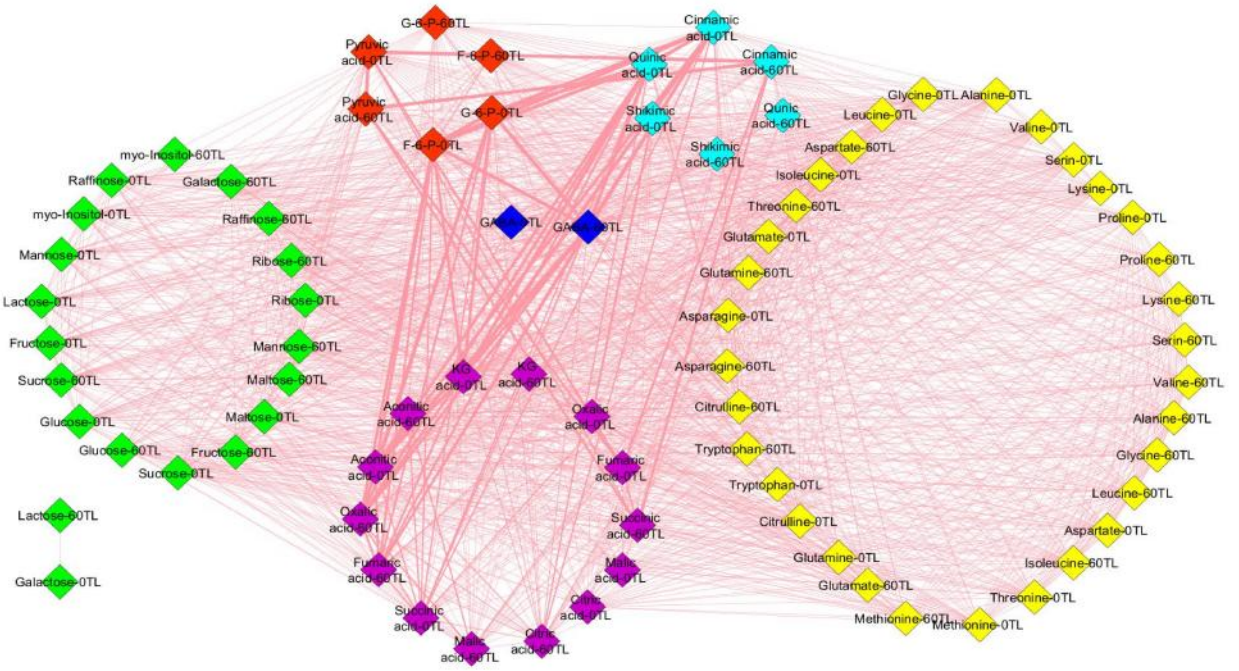

Figure 4.6. Changes in *Ca*754 and *Ca*J-19 metabolite interactions as a result of drought stress conditions at 0TL and 60TL. Nodes correspond to metabolites and edges between nodes represent Pearson correlations with $r \geq 0.8$. Yellow, green, red, pink, blue and aqua colors represent amino acids, sugars, glycolysis pathway, tricarboxylic acid pathway, γ-aminobutyric acid pathway and shikimic acid pathway metabolite groups, respectively. TL refers to the time line.

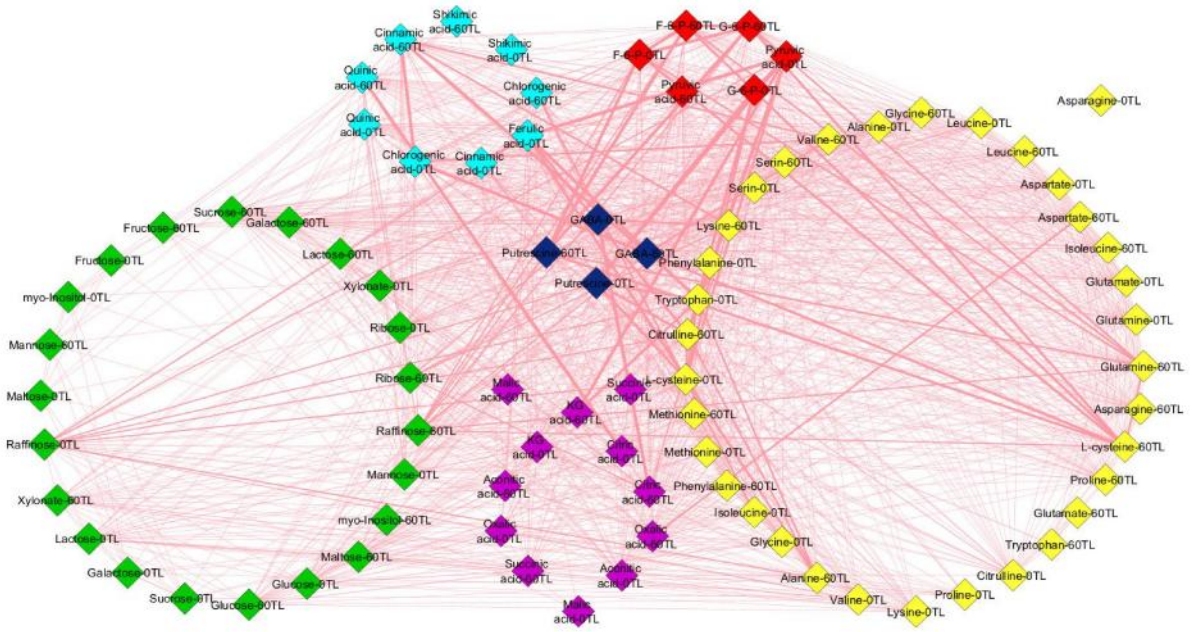

Figure 4.7. Changes in *Ca*74110 and *Ca*74112 metabolite interactions due todrought stress conditions at 0TL and 60TL. Nodes correspond to metabolites and edges between nodes represent Pearson correlations with $r \geq 0.8$. Yellow, green, red, pink, blue and aqua colors represent amino acids, sugars, glycolysis pathway, tricarboxylic acid pathway, γ-aminobutyric acid pathway and shikimic acid pathway metabolite groups, respectively. TL refers to the time line.

4.3.7.3. PCA analysis

PCA results demonstrate an obvious metabolite accumulation and genotype category in the course of the drought stress exposition. The first principal component (PC1) and second principal component (PC2) represent 86.77% and 8.39% of the PCA, respectively. PC1 was dominated by

by the metabolites glucose, maltose, tryptophan, galactose, lactose, lysine, methionine, leucine, valine, ribose, isoleucine, pyruvic acid, and serine; whereas, maltose, fumaric acid, malic acid, ferulic acid, α-Ketoglutaric acid, chlorogenic acid, valine, shikimic acid, and succinic acid were major contributors for separation along PC2 (see Figure 4.8).

In order to understand the drought induced responsive metabolites, the metabolite's up- and down-accumulation score value were considered for the analysis in each genotype, and the score value of PC1 (86.77%) was taken as the weight. As a result of this, significantly (p<0.001) the most responsive metabolite PC1 score values were identified in glucose (11.96 PCA score value), maltose (9.79 PCA score value), tryptophan (8.08 PCA score value), galactose (5.47 PCA score value), lactose (4.50 PCA score value), lysine (4.42 PCA score value), methionine (3.23 PCA score value), leucine (2.99 PCA score value), valine (2.94 PCA score value), ribose (2.71 PCA score value), isoleucine (2.70 PCA score value), pyruvic acid (2.70 PCA score value), and serine (2.60 PCA score value) (Appendix 15).

Similarly, following the same protocol, to briefly examine the drought tolerance capacity of the genotypes, the genotypes-against the respective water treatments- score values were taken in to consideration. The result showed that the PC score value of the genotypes under drought stressed conditions was significantly (p<0.05) higher than those under well watered conditions. However, among the coffee genotypes under drought stressed conditions, significantly the highest PC1 score values were identified by the relatively tolerant genotypes of *Ca*74112 (0.972 PCA score value) and *Ca*74110 (0.977 PCA score value) which were followed by the sensitive genotypes of *Ca*J-19 (0.803 PCA score value) and *Ca*754 (0.679 PCA score value) (Appendix 16).

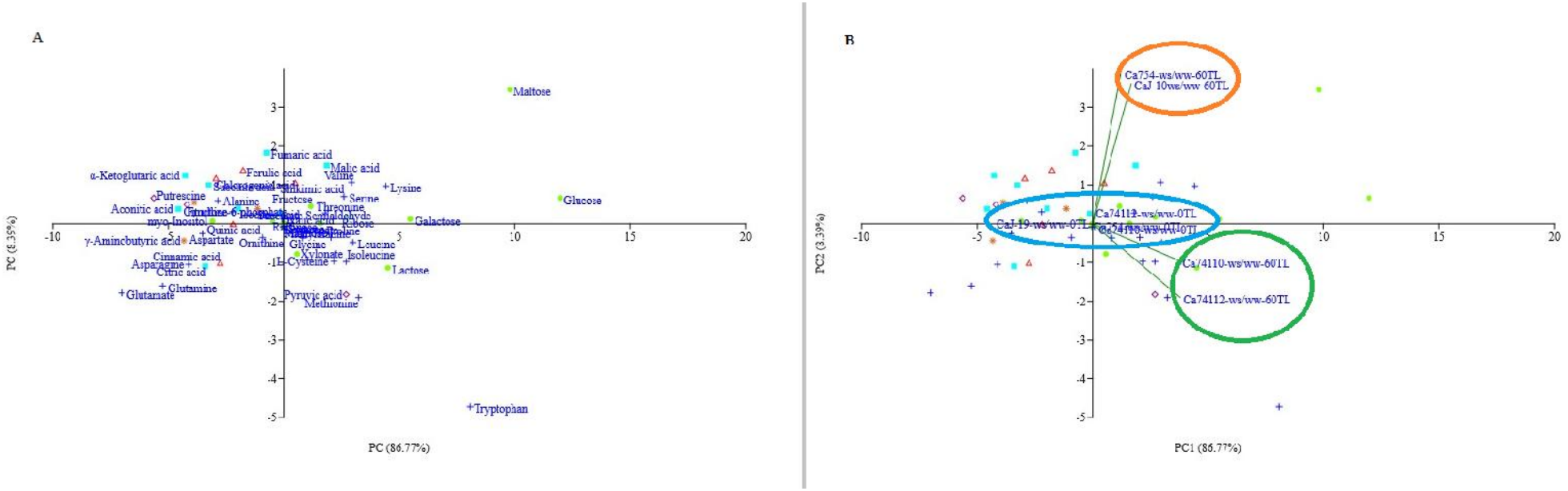

Figure 4.8. Principle component analysis (PCA) plot (x – first component, y – second component) plot indicating (**A**) the row labels of each metabolite, and (**B**) the biplot of *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112 coffee genotypes, based on GC/MS analysis, at 0 and 60 days after drought begins. Sugars (orange, dot), amino acids (blue, plus), TCA (aqua blue, square), glycolysis (pink, diamond), GABA shut (brown, star), and shikimik pathway (red, triangle).

4.4. Discussion

4.4.1. Genotypic variability and physiological responses

The physiological impact of drought stress on plants is often manifested in restricted growth and developmental limitations due to the scarcity of water (Konieczna *et al*., 2023). Coffee plants, both at the seedling or mature stages, exhibit high sensitivity to soil moisture levels, profoundly affecting their subsequent growth and development (Gugliuzza *et al*., 2020; Habtamu Chekol *et al*., 2023). Consistent with the findings of da Silva *et al*. (2022), our study noted a significant decline in the growth performance of coffee plants under drought stress, reflected in reduced shoot length and lower fresh and dry biomass.

Notably, after a 60-day drought stress period, relatively higher shoot length and increased fresh and dry biomass were observed in the relatively tolerant genotypes *Ca*74110 and *Ca*74112 compared to the sensitive genotypes *Ca*754 and *Ca*J-19. Under drought stress, any decrease in turgor pressure and water potential can impede cell division, expansion, and elongation, leading to reduced leaf area, smaller leaf size, and ultimately lower photosynthetic rates by limiting CO_2 assimilation (da Silva *et al*., 2022; Habtamu Chekol *et al*., 2023; Konieczna *et al*., 2023). Studies by DaMatta *et al*. (2007) and Wei *et al*. (2016) have highlighted the impact of drought stress on diminishing shoot and root growth in coffee and *Lycium barbarum* plants, respectively. However, previous research by Dias *et al*. (2007) and Habtamu Chekol *et al*. (2023) suggest that in tolerant genotypes, enhanced growth responses are linked to water conservation mechanisms that enable coffee plants to sustain cell division and elongation processes. Studies by Caine *et al*. (2019) and Xiong *et al*. (2022) on rice and oak plants, respectively, also support the notion that limited water availability triggers metabolic responses favoring cellular division. This, in turn, favoring the development of dermal tissue, ground tissue, and vascular tissue, essential

components contributing to the plant's adaptation to drought stress. Shoot growth and development serve as key indicators of a plant's response to drought stress and are often considered key parameters in assessing a plant's drought tolerance (Cai *et al*., 2007). Similar with the observations of Mirian *et al*. (2006), our results indicate that the relatively tolerant genotypes *Ca*74110 and *Ca*74112 maintain higher growth metrics—such as shoot length, fresh and dry biomass, and specific leaf area—more effectively than the sensitive genotypes *Ca*754 and *Ca*J-19 under drought stress conditions.

4.4.2. Relative water contents, gas exchange and pigments variations among coffee genotypes towards drought stress

In the current study, drought stress significantly impacted various physiological parameters of coffee genotypes, notably reducing leaf relative water content, net assimilation rate, stomatal exchange, and chlorophyll pigments compared to well-watered conditions. Similar reductions in these parameters under drought stress were reported in other studies on cowpea (Hayatu *et al*., 2014), coffee (da Silva *et al*., 2022; Habtamu Chekol *et al*., 2023), and other tolerant crops (Pirzad *et al*., 2011). The tolerant coffee genotypes (*Ca*74110 and *Ca*74112), displayed higher relative water content even under drought stress, aligning with findings in potato genotypes reported by Soltys-Kalina *et al*. (2016).

Drought stress often leads to decreased photosynthesis assimilation rates, stomatal conductance, and affecting gaseous exchange parameters (Xiong *et al*., 2022; Konieczna *et al*., 2023). Similarly, tolerant coffee genotypes exhibited better physiological performances in these parameters compared to sensitive genotypes under drought stress (Dias *et al*., 2007; Habtamu Chekol *et al*., 2023). The reduction in photosynthesis rate under drought is usually associated

with stomatal closure and decreased internal CO_2 concentrations, impacting CO_2 fixation and pigment synthesis (da Silva *et al*., 2022; Wang *et al*., 2018; Zhang *et al*., 2021). This decline in photosynthesis rate, along with reduced stomatal conductance often leads to diminished pigment synthesis (Taiz and Zeiger, 2010). Tolerant genotypes (*Ca*74110 and *Ca*74112) maintained higher pigment contents even under drought stress compared to sensitive genotypes (*Ca*754 and *Ca*J-19). Drought stress often affects the structure and function of photosynthetic pigments by damaging thylakoid membranes and reducing the activity of essential enzymes like RUBISCO (Mafakheri *et al*., 2010). This stress-associated decline in chlorophyll content is observed in various crops, indicating damage to light-harvesting complex proteins, impacting photon absorption and electron transport (Nikolaeva *et al*., 2010; Manivannan *et al*., 2007). Perhaps, relatively drought-tolerant genotypes (*Ca*74110 and *Ca*74112) might possessed better protective mechanisms against chlorophyll degradation enzymes than sensitive genotypes (*Ca*754 and *Ca*J-19).

4.4.3. Drought stress causes variability in metabolite alteration among coffee genotypes

Drought stress triggers significant changes in the biosynthesis and transport of primary and secondary metabolites, orchestrating adjustments in plants' physiological and biochemical processes (Rabara *et al*., 2017). Differentiating between tolerant and sensitive behaviors, plants respond diversely to shifting soil moisture regime (da Silva *et al*., 2022). Tolerant plants usually sustain metabolic processes and defense responses, whereas sensitive ones operate in the opposite manner (Isah, 2019).

In our study, we focused on sugars, amino acids, and organic acids synthesed in different pathways like glycolysis, GABA shut, TCA cycle, and shikimic path. After a 60-day drought stress period, relatively tolerant genotypes (28 in *Ca*74110 and 29 in *Ca*74112) up-accumulated more metabolites compared to sensitive genotypes (25 in *Ca*754 and 21 in *Ca*J-19). Conversely, sensitive genotypes (18 in *Ca*754 and 22 in *Ca*J-19) down-accumulated more metabolites than relatively tolerant ones (17 in *Ca*74110 and 16 in *Ca*74112). This reflects significantly higher metabolite up-accumulation in the relatively tolerant *Ca*74112 and *Ca*74110 genotypes compared to sensitive *Ca*754 and *Ca*J-19 genotypes ($p<0.01$).

According to Fabregas and Fernie (2019), organic biomolecules like sugars, amino acids, and others play pivotal roles in osmotic adjustment during drought stress by regulating vacuolar osmotic potential. Kapoor *et al*. (2020) also noted that these metabolite responses to drought vary not only between species but also among genotypes and different parts of the plant. The PCA analysis further revealed distinct separation and clustering between the relatively tolerant and sensitive coffee genotypes, indicating diverse mechanisms of metabolite alteration in response to drought stress. Previous findings by Rodrigues *et al*. (2021) on coffee plants and Hochberg *et al*. (2013) on grapevines suggest that prolonged drought stress triggers adjustments in various metabolites, enhancing the plants' resilience to drought. Similarly, Xiong *et al*. (2022) on Quercus species indicate that during drought stress, metabolite alterations are more pronounced in tolerant genotypes, providing enhanced resistance to manage plant growth and development.

Sugars are highly sensitive metabolite classes to drought stress and extensively studied (Lozano-Elena *et al*., 2022). Profiling 12 sugars showed increased levels across all genotypes under drought stress condition, notably higher in relatively tolerant *Ca*74110 and *Ca*74112, particularly in glucose, maltose, galactose, lactose, and ribose, compared to sensitive *Ca*754 and

*Ca*J-19. However, in the current study myo-inositol concentration decreased universally, indicating its drought sensitivity. These sugars play key roles in osmotic adjustments, membrane stability, and maintaining leaf water content during drought stress (Fabregas and Fernie, 2019). Previous studies by Urano *et al*. (2009), Krasensky and Jonak (2012), Fabregas *et al*. (2018), Ogbaga *et al*. (2016), and Pires *et al*. (2016) align with our findings, demonstrating increased fructose, glucose, raffinose, and other sugar levels during drought stress in various plants. Additionally, similar to our observations, Urano *et al*. (2009) also noted a reduction in myo-inositol content under drought conditions.

The study identified 19 amino acids, showing up-accumulation in certain amino acids across all genotypes, particularly pronounced in relatively tolerant *Ca*74110 and *Ca*74112, including tryptophan, L-Cysteine, lysine, methionine, valine, leucine, isoleucine, serine, and proline compared to sensitive *Ca*754 and *Ca*J-19. However, alanine, aspartate, glutamate, glutamine, and asparagine decreased universally. During drought stress, amino acids usually act as osmolytes and scavengers of reactive oxygen species, influencing cellular functions (Ahanger *et al*., 2018). Proline accumulation correlates with drought tolerance (Singh *et al*., 1972; Szabados and Savoure, 2010), as demonstrated by various studies including Konieczna *et al*. (2023), Zhang *et al*. (2011), and Joshi *et al*. (2010). Decreases in certain amino acids during drought stress might be due to redirect metabolic activities towards proline biosynthesis (Lehmann *et al*., 2010). Enhanced protein degradation or inhibition of biosynthesis presumably contributes to amino acid increases during prolonged drought (Pires *et al*., 2016). Amino acids linked to pyruvate metabolism increase might be due to their involvement in gluconeogenesis to alleviate transamination products (Zhang *et al*., 2017). The correlation-based network analysis also demonstrated heightened coordinated metabolic activities in relatively tolerant coffee genotypes,

showcasing their resilience to drought stress. Hochberg *et al*.'s (2013) finding in grapevines research supported this, indicating that prolonged drought stress can boost metabolic network density. Likewise, Sanchez *et al*. (2011) observed increased network connectivity in Lotus genotypes facing salt stress, aligning with these findings.

Among the identified 8 tricarboxylic acid (TCA) cycle intermediates, mostly marked reduction levels across all genotypes were apparent, particularly higher in relatively tolerant *Ca*74110 and *Ca*74112 (malic acid, oxalic acid) compared to sensitive *Ca*754 and *Ca*J-19. TCA cycle metabolites' responses in drought stress remain less pronounced than sugars and amino acids. Araujo *et al*. (2012) also noted limited alterations in the TCA cycle during drought stress, similar to this study. Fabregas *et al*. (2018) demonstrated analogous changes in Arabidopsis, linking increased malic acid and oxalic acid levels to suppressed malate dehydrogenase, aiding nutrient uptake and intracellular ionic regulation under drought conditions. The increase in malic acid level and oxalic acid is associated with decreased sink tissue utilization due to malate dehydrogenase suppression (Rzepka *et al*., 2009). Yang *et al*. (2018) also reported declines in citrate, succinate, α-ketoglutarate, and fumarate in drought-stressed maize kernels, aligning with this study's findings.

The study examined 11 metabolites from glycolysis, GABA, and shikimic biosynthetic pathways, observing increased levels universally in all genotypes, notably more pronounced in relatively tolerant *Ca*74110 and *Ca*74112 (pyruvic acid, shikimic acid) compared to sensitive *Ca*754 and *Ca*J-19. However, fructose-6-phosphate, glucose-6-phosphate, γ-aminobutyric acid, succinate semialdehyde, and putrescine exhibited reduced levels across all genotypes. Consistent with this study, Rabara *et al*. (2017) also reported decreased glucose-6-phosphate and fructose-6-phosphate in tobacco and soybean leaves under drought stress. Guo *et al*. (2018) observed a

declining trend in GABA shunt metabolites in wheat under drought. As per Kinnersley's review (2000), γ-aminobutyric acid (GABA) levels increased in response to drought stress in various plant species.

4.5. Conclusion

This study explores drought stress impact on Arabica coffee, focusing on physiological and metabolic changes in genotypes with varying tolerance. Notably higher shoot growth, biomass, CO_2 assimilation, pigments were evident in tolerant genotypes (*Ca*74110 and *Ca*74112) than sensitive group under drought stress. Metabolite profiling reveals elevated glucose, maltose, amino acids, and organic acids, suggesting enhanced compatible solutes crucial for drought tolerance, with these changes more pronounced in drought-tolerant genotypes, showcasing their resilience. In this context, delving further into gene expression presents a promising avenue for the development of drought-tolerant coffee genotypes aimed at achieving sustainable yields and productivity.

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5. CHAPTER FIVE

General Discussion, Conclusion and Recomendation

5.1. General Discussion

Under the ongoing climate change scenarios, abiotic stresses are a serious threat to global crop security and plant production sustainability (Haggar and Schepp, 2012). Coffee plants are one of the sensitive species, and highly vulnerable to environmental stresses (Seleiman *et al*., 2021). Among the abiotic stresses, drought stresses are gaining attention due to their adverse effect on coffee growth and development and a significant reduction in yield and biomass causing global coffee bean insecurity (Bilen *et al*., 2023). Drought stress affects the physiological and developmental activities of coffee plants in their life cycle ranging from germination events to maturity phase (Moat *et al*., 2017).

Coffee seed traits are usually influenced by both internal and external factors. One of the key seed traits associated with germination is the moisture content, which is vital for determining the germination potential of coffee seeds (Finch-Savage and Leubner-Metzger, 2006). Similarly, elongated and heavier seeds provide efficient germination capacity and improve the seed's potential to tolerate drought conditions (Steinbrecher and Leubner-Metzger, 2017). Coffee seeds, like the relatively tolerant genotypes (i.e., *Ca*74112, *Ca*74110, and *Ca*74140), with higher moisture content and larger, possess relatively higher content of hydrophilic molecules in the endosperm, get hydrated, and allow more oxygen to enter the embryo and activates aerobic respiration, triggers the activation of hydrolyzing enzymes that catalyze the hydrolysis of food reserves in the endosperm, resulting in the transformation of the quiescent embryo to a metabolically active one, and germinate faster even under limited water availability or in drought stress conditions (DaMatta *et al*., 2007; Silva *et al*., 2008; Weitbrecht *et al*., 2011; Steinbrecher and Leubner-Metzger, 2017). The early completion of germination events in these genotypes

may allow the seedling of tolerant genotypes to grow rapidly, promote seedlings' plasticity, and withstand a resource-limited environment (Rosa *et al*., 2010; Voegele *et al*., 2012).

Apart from coffee germination, drought stress is well known to reduce the growth and physiological processes of adult *C. arabica* (Brown and Mayer, 1988; Weitbrecht *et al*., 2011). Under drought stress, there is a decline in turgor pressures that leads to a reduction in cell division, elongation, and expansion, and consequently decreases the growth of shoot and root, and reduces internal water exchange, photosynthesis, stomatal conductance and transpiration and pigment synthesis, and facilitates relative cell injury (Giorgini and Comoli, 1996; Steinbrecher and Leubner-Metzger, 2017).

The present study showed a significant reduction in terms of shoot and root growth and development identified when the coffee genotypes were grown under drought stress than well-watered conditions. According to Cai *et al*. (2007), Razmjoo *et al*. (2008), and Oguz *et al*. (2022), a shortage of water content or turgor pressure, leads to disturbing the plant water relations and reduces the entry of macro and micronutrients, and creates a disruption of the cellular mitosis process that greatly restricts and reduces cell division, cell elongation, and differentiation, and consequently limited and reduced the growth and development of leaf number, leaf size, leaf area, root number, root length, root volume, as well as the reduction of fresh and dry biomass. However, the study revealed that, by withstanding the drought stress conditions, relatively tolerant genotypes, showed much better efficiency in terms of stem height, collar diameter, leaf number, leaf area, root length, root volume, root number, and higher relative growth rate, indicating more water conservation characteristics to tolerate the drought stress, therefore, maintaining a more favorable internal water status. Under drought stress, those genotypes with long and advanced root systems can easily uptake water and nutrients and have

successful plant growth and development avoiding drought stress (Ronchi *et al*., 2007; Mateva *et al*., 2022). According to DaMatta (2004), Dias *et al*. (2007), Poorter *et al*. (2011), and Soizig *et al*. (2021), as a result of drought stress-induced reduction of shoot and root growth and development, coffee plants also face a significant reduction of fresh and dry biomasses, and show a shift in biomass allocation from leaves and stems to roots.

Drought stress leads to a decrease in plants' relative water content and water potential where the reduction is low in tolerant genotypes (Hayatu *et al*., 2014). Similar to this study, Soltys-Kalina *et al*. (2016) reported the reduction of the relative water content of potatoes under drought stress conditions, but the report also identified that the tolerant genotypes are characterized by having higher relative water content than the sensitive genotypes. Genotypic variation of water potential may be attributed to differences in the ability to absorb more water from the soil and the ability to reduce water loss through stomata (Siddique *et al*., 2000). Drought stress reduces gas exchange parameters (net photosynthesis rate, transpiration rate, stomatal conductance, and water use efficiency) by influencing the indices of gaseous exchange parameters regardless of the genotype (DaMatta, 2003; Blackman *et al*., 2009; Bhusal *et al*., 2021). In tolerant genotypes (*Ca*74112 and *Ca*74110), as the drought-stress intensifies, the water use efficiency and CO_2 assimilation rate is relatively higher than in the sensitive genotype. Water deficit leads to the reduction of photolysis reaction in the photosystem that minimizes the formation of free hydrogen ions and electrons during electron transport chain systems, that in turn inhibits adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) production, that will be utilized in the dark reaction of photosynthesis (Nikolaeva *et al*., 2010; Zargar *et al*., 2017). In addition, drought stress-induced abscisic acid (ABA) promotes stomatal closure to conserve the remaining internal water from loss consequently lowering the internal

CO_2 concentrations in the mesophyll and decreasing CO_2 fixation by inhibiting the synthesis of ribulose bisphosphate (RUBP) (Chaves *et al*., 2009; Zhang *et al*., 2021). Similar to our findings, Dias *et al*. (2007), and da Silva *et al*. (2022), reported that relatively tolerant coffee genotypes have the potential to display much-improved water use efficiency, photosynthesis assimilation rate, stomatal conductance, and transpiration rate, unlike the sensitive genotypes, even under drought stress conditions. DaMatta *et al*. (2008) reported that coffee genotypes with higher water use efficiency have higher assimilation rate and relative growth rate. Drought stress also influences the structure and functions of photosynthetic pigments by damaging the complex protein structures of the thylakoid membranes and decreasing the activities of RUBISCO enzymes (Taiz and Zeiger, 2010). The reduction is associated with the damage that occurred in the pigments of the light-harvesting complex proteins which directly impacts the photon absorption and electron transport chain (Catalina *et al*., 2010; da Silva *et al*., 2022; Maxiselly *et al*., 2022). Jaleel *et al*. (2009) also reported that intensified drought-stress causes the degradation of photosynthetic pigments, damage to the membrane system, and the reduction of synthetase activity. Kirnak *et al*. (2001) associated the reduction of chlorophyll concentrations with increased electrolyte leakage due to softening and breakage of the cell wall. Since the amount of electrolyte leakage is a function of membrane permeability, the degree of cell damage resulting from water deficit can be assessed by measurement of electrolytic conductance in terms of cell membrane stability and relative cell injury (Kumar *et al*., 2012; Rehman *et al*., 2016). Similar to the findings of Quilambo (2004), Suzuki *et al*. (2014), Pandey *et al*. (2015), and ElBasyoni *et al*. (2017), the result of this study revealed that relatively tolerant genotypes showed high cell membrane stability with increased membrane stabilizers, i.e., heat-shock proteins, and saturated lipids, indicating a high level of drought stress tolerance response.

Drought stress usually prompts up- and down-accumulation and profound changes in biosynthesis and transport of metabolites, to regulate a changing physiological and biochemical process (Rabara *et al*., 2017). According to Fabregas and Fernie (2019) and Lozano-Elena *et al*. (2022), during drought stress, plant's organic molecules including sugars, amino acids, tri-carboxylic acid, glycolysis, gamma amino butyric acid shut and shikimic acids, are known to play important roles during osmotic adjustment through regulating the vacuolar osmotic potential, and Kapoor *et al*. (2020) also stated that such metabolite responses vary between plant genotypes. Similar to the findings of Urano *et al*. (2009), Krasensky and Jonak (2012), Ogbaga *et al*. (2016), Pires *et al*. (2016), and Fabregas *et al*., (2018), in this study, increased sugar levels were observed in all genotypes, but in the relatively tolerant genotype, under drought stress conditions, unlike myo-inositol, the accumulation of glucose, tryptophan, maltose, galactose, L-cysteine, lactose, lysine, methionine, leucine, pyruvic acid, ribose, and other amino acids and organic acids, are more expressed and profiled than the sensitive genotypes.

Sugar accumulation during drought stress promotes osmotic adjustments, maintains leaf water content, prevents oxidation and dehydration of cell membranes, and increases drought tolerance capacity (Fabregas and Fernie, 2019). Similar to the findings of Szabados and Savoure (2010), Sanchez *et al*. (2012), Zhang *et al*. (2011), and Ahanger *et al*. (2018), the current study showed the up accumulation of tryptophan, L-cysteine, lysine, methionine, valine, leucine, isoleucine, serine, and proline. According to Pires *et al*. (2016), the amino acids up-accumulation was associated with enhanced protein degradation or the inhibition of protein biosynthesis because plant growth was inhibited with prolonged drought stress, and the amino acids serve as osmolytes to balance cellular osmotic potential and as scavengers of reactive oxygen species. Furthermore, similar to Yang *et al*. (2018), Kinnersley (2000), Rabara *et al*. (2017), and Guo *et*

al. (2018), in this study, malic acid, oxalic acid, pyruvic acid, and shikimic acid, are more expressed and profiled more in the relatively tolerant coffee genotypes. Apart from sugars and amino acids, intermediate metabolites are not well described, however, according to Araujo *et al*. (2012), they remained relatively unaltered in drought stress, but with limited accumulation responses. The limited accumulation is related to the lack of utilization in sink tissue because of the suppression of various specific enzymes, and as a result, it supports nutrient uptake and keeps intracellular ionic regulation to overcome drought stress conditions (Rzepka *et al*., 2009).

5.2. Conclusion

Drought stress is a worldwide problem, constraining global coffee production and quality, and the ongoing global climate change has made this situation more serious. The timing, duration, severity, and speed of stress undoubtedly play an important role in determining a coffee's response to a lack of water. The response of coffee to drought stress at different growth stages is an important criterion for the development of genotypes with high drought stress tolerance. The response to drought stress occurs as a result of the cooperation of molecular, biochemical, physiological, and morphological mechanisms. Each of these mechanisms is very complex to be considered separately. Focusing on the differences in the activation and regulation of these mechanisms during important development stages of the plant may lead to new approaches, as in this study.

In this study, it is found that the exposure of coffee seeds, seedlings, and adult plants to drought stress reduces germination rate, growth and development, and physiological processes. The result also revealed that drought stress leads to the alteration of various metabolites of the coffee genotypes. It is concluded that the germination rate, growth, and physiological and biochemical, molecular processes of coffee genotypes were impaired by drought stress during seeds, seedlings, and adult plants' developmental stages. The present study revealed that the coffee plants belonging to different genotypes had notable differences in the period needed to finish their germination and post-germination stages of growth. The relatively tolerant genotypes completed each stage of germination and post-germination seedling development faster and thus generated more robust seedlings compared to the moderately sensitive and sensitive groups. Drought stresses hampered the morphological traits such as stems, leaves, roots, biomass developments, and relative growth rate, and impaired physiological performances such as

relative water content, water potential, water use efficiency, carbon assimilation, stomatal conductance, transpiration, pigment contents, and cell membrane stability of *C. arabica* genotypes, while a significant boost in stomatal densities was also observed in all genotypes. Additionally, key metabolites from sugars, amino acids, tri-carboxylic acid, γ-aminobutyric acid, glycolysis, and shikimic pathways were more accumulated at high concentrations in relatively tolerant genotypes and can be concluded to be a mechanism used to tolerate drought stress episodes. The result obtained in this study provided countless information that could be used for building a coffee model coupling aquaporin gene and their expression under drought stress environmental conditions. The fitness of coffee plants submitted to drought stress events depends on the adequacy of germination rate, growth, and physiological and biochemical, molecular, and consequently, these aspects should be accounted for in breeding programs aimed at improving drought tolerance in coffee plants.

5.3. Recommendation

- A key message from this research resides in the fact that evaluation of other *C. arabica* genotype's performance under drought stress conditions should combine analyses of germination, morpho-physiological, and biochemical processes at seed, seedling, and adult plant levels.
- The analysis of seed traits should be used to maximize the germination of *C. arabica* seeds and improve the growth and development of this economically important crop, and the inherent qualities of the seeds are critical for their germination capability and post-germination developments of seedlings and the consequent adult coffee plants.
- The analysis of morpho-physiological, biochemical, and metabolite trails is critical and vital for screening and developing drought-stress-tolerant *C. arabica* genotypes to be used for under drought-intense coffee growing areas.
- To fulfill the demand for coffee consumption under the ongoing drought-stress conditions, a possible approach for promoting drought-stress tolerance *coffee* genotypes is mandatory by identifying and adjusting the expression levels of genes of drought-tolerant coffee plants using biotechnological and genetic engineering approaches, and such approaches for drought tolerance development program will have a massive impact on sustainable coffee bean yields and productivity to address the demand of coffee consumption.
- Aquaporins, as well as flowering gene expression study, should be given priority, thus factors controlling aquaporins and flowering genes would be identified, and the coffee research sector could manipulate that gene capable of regulating coffee production under

drought stress conditions, as a result, an improved coffee management systems will be developed.

- Stakeholders should be made aware of the importance of assessing coffee-associated ecosystem services, related to drought stress-impacted areas, to target investments and prioritize actions, including selection of coffee genotypes capable of withstanding drought stress, to increase the sustainability and resilience of coffee systems worldwide.
- Finally, the Ethiopian Agricultural Research Institute (EARI) should cooperate with the World Coffee Research (WCR) institute to support and improve coffee breeding programs in Ethiopia, to unlock center for screening and developing drought stress tolerant genotypes, with the potential to grow and develop under water deficit environmental conditions.

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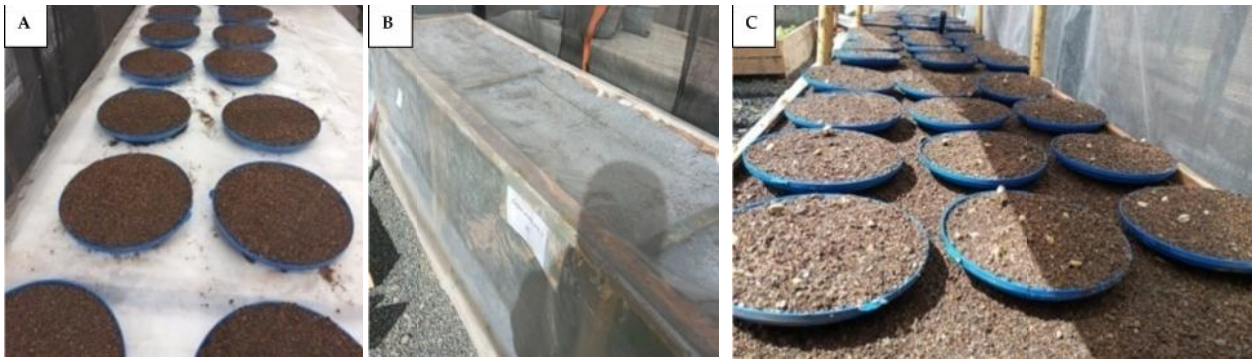
APPENDICES

Appendix 1. Seeds of the nine *C. arabica* genotypes used in this study. Pictures were taken using Sony Alpha A7RIV.

Appendix 2. Mean values and SD of pre-germination parameters of the coffee genotypes.

Varities	Fw, g	SD ±	Dw, g	SD ±	Mc-dry, %	SD ±	Sl, mm	SD ±	Sw, mm	SD ±	SA, mm^2	SD ±	SV, mm^3	SD ±	SA/SV	SD ±
*Ca*754	0.161	0.002	0.149	0.002	8.05	0.175	7	0.213	7.4	0.224	51.800	2.46	200.604	12.340	0.258	0.01
*Ca*J19	0.204	0.002	0.184	0.002	10.87	0.190	7.3	0.213	7.3	0.133	53.290	2.92	203.586	9.450	0.262	0.03
*Ca*Geisha	0.186	0.001	0.167	0.002	11.38	0.160	7.2	0.180	7.3	0.153	52.560	1.75	200.797	11.670	0.262	0.01
*Ca*J21	0.200	0.003	0.179	0.002	11.73	0.255	7.6	0.213	7.1	0.133	53.960	2.25	200.497	10.030	0.269	0.02
*Ca*74165	0.173	0.001	0.154	0.002	12.34	0.135	8.6	0.306	7.4	0.100	63.640	1.75	246.457	8.470	0.258	0.02
*Ca*74158	0.193	0.001	0.171	0.002	12.87	0.140	8.8	0.291	7.2	0.153	63.360	1.85	238.740	10.320	0.265	0.01
*Ca*74110	0.165	0.003	0.144	0.001	14.58	0.190	8.9	0.233	7.1	0.153	63.190	2.56	234.793	9.240	0.269	0.03
*Ca*74112	0.197	0.001	0.172	0.003	14.71	0.195	9.6	0.267	7	0.233	67.200	1.6	246.176	10.240	0.273	0.01
*Ca*74140	0.200	0.002	0.174	0.001	14.89	0.150	9.9	0.233	6.9	0.100	68.310	1.89	246.667	9.330	0.277	0.02

Appendix 3. The shade and greenhouse for the germination of *C. arabica* seeds: (A) washed and autoclave-sterilized sand arranged for sowing the coffee seeds in a plastic tray with the hole at the base, (B) poly-propagator that provides efficient microclimatic conditions for the germination of the coffee seeds, and (C) upward growth of germinant, during the study period. Pictures were taken using Sony Alpha A7RIV.



Appendix 4. Representative example of *C. arabicas* and germination process of the nine genotype seeds: (A) early stage (maximum 26.0±2.31 days), (B) matchstick stage (max. 32.0±2.09 days), (C) butterfly stage (max. 46.0±2.23 days), and (D) transplanting stage (max. 53.2±3.86 days). Pictures were taken using Sony Alpha A7RIV.

Appendix 5. Mean values and SD for germination perameters of the coffee genotypes.

Genotypes	GP, %	SD ±	MGT	SD ±	CV_t	SD ±	CVG	SD ±	GI	SD ±	U	SD ±	Z	SD ±	MDG	SD ±	Pv	SD ±	Gv	SD ±	GRI	SD ±
*Ca*754	35	2.67	17.52	0.27	2.92	0.03	5.71	0.850	1.20	0.30	1.00	0.14	0.48	0.04	1.40	0.19	1.94	0.37	2.72	0.89	1.99	0.65
*Ca*J19	45	2.21	16.27	0.21	3.85	0.02	6.15	0.620	2.03	0.42	1.32	0.12	0.42	0.02	2.20	0.10	3.24	0.47	7.12	0.11	3.38	0.43
*Ca*Geisha	55	2.46	16.09	0.36	5.00	0.03	6.21	0.560	2.06	0.51	1.49	0.14	0.44	0.05	2.20	0.06	3.06	0.18	6.72	0.97	3.43	0.76
*Ca*J21	55	2.92	13.78	0.32	3.07	0.04	7.26	0.870	1.96	0.11	0.76	0.10	0.64	0.04	1.80	0.17	3.21	0.22	5.79	0.88	3.27	0.88
*Ca*74165	60	1.75	13.33	0.25	4.74	0.03	7.50	0.980	2.71	0.28	1.33	0.16	0.41	0.03	2.40	0.08	4.29	0.44	10.29	1.10	4.51	0.16
*Ca*74158	65	1.85	14.15	0.35	3.81	0.04	7.07	1.210	2.76	0.48	1.14	0.09	0.53	0.02	2.60	0.05	4.33	0.53	11.27	1.24	4.60	0.53
*Ca*74110	75	1.56	10.07	0.26	18.14	0.02	9.93	1.500	4.61	0.62	2.61	0.13	0.20	0.03	3.00	0.19	5.50	0.45	16.50	1.36	7.69	0.75
*Ca*74112	80	1.74	9.50	0.24	20.94	0.05	10.53	1.820	5.25	0.26	2.61	0.18	0.19	0.04	3.20	0.18	6.11	0.44	19.56	1.04	8.75	1.06
*Ca*74140	90	1.44	11.61	0.24	10.10	0.04	8.61	1.050	4.70	0.51	2.17	0.18	0.25	0.03	3.60	0.15	6.54	0.27	23.54	1.18	7.83	1.01

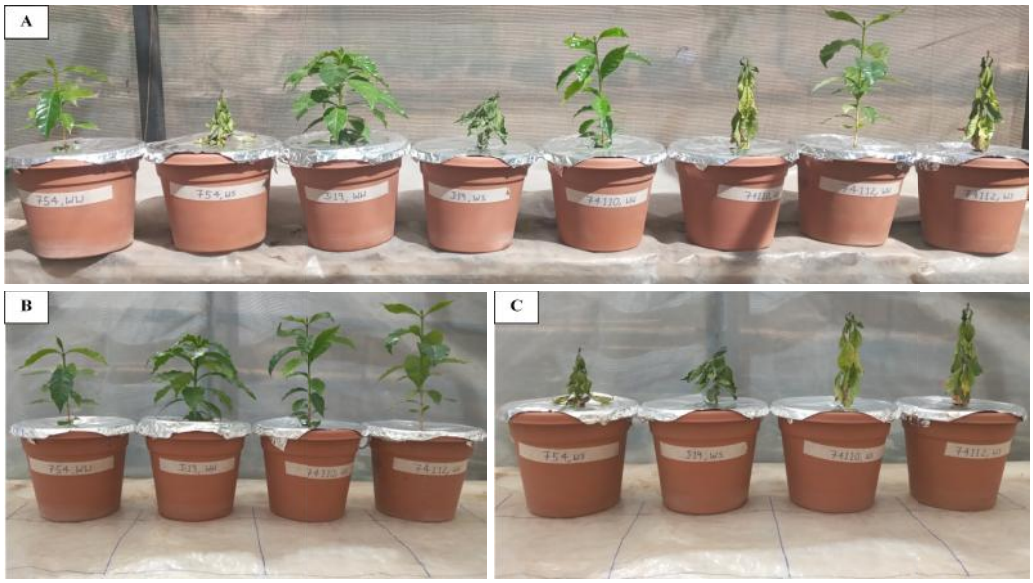
Appendix 6. Transplanting the *C. arabica* genotypes (A) from the sand media, (B) pulling up the genotypes without root damage, (C) the initial seedling, (D) initial seedling after transplanting, (E) seedlings at the age of 6 leaf pairs (6 month sold), (F) at the start of the experiment when genotypes developed 7–8 leaf pairs (8–9 months old). Pictures were taken using Sony Alpha A7RIV.

Appendix 7. Mean values and SD of stem height (cm), stem diameter (mm), leaf number, leaf area (cm^2), root length (cm), root number, and root volume (cm^3) of the four coffee (*Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112) genotypes.

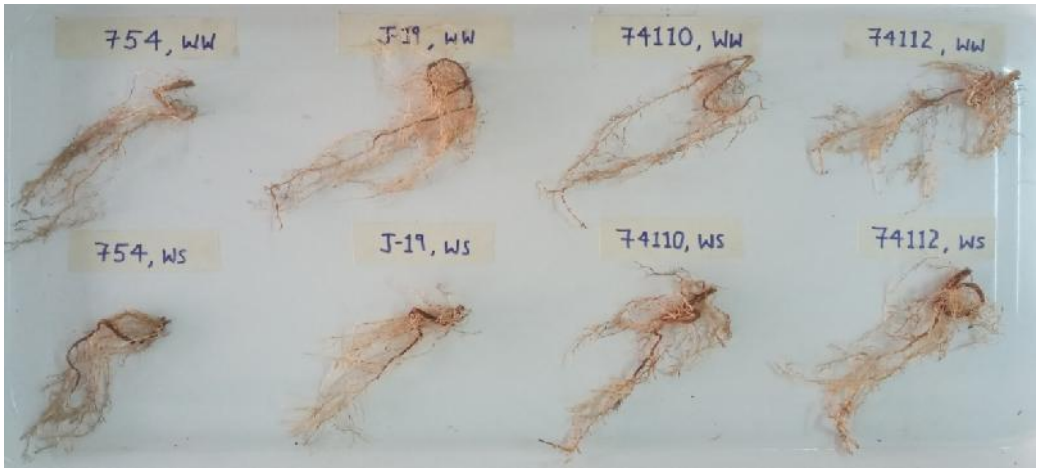
Vari.	DADB	*Ca*754				*Ca*J-19				*Ca*74110				*Ca*74112			
		ww		*ws*		*ww*		*ws*		*ww*		*ws*		*ww*		*ws*	
		M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Stem height	**0**	3	0.196	3	0.235	4.9	0.0913	4.88	0.214	7.1	0.204	7	0.129	9.28	0.075	9.75	0.05
	10	4.25	0.132	4.2	0.314	6.13	0.0479	6.12	0.202	8.3	0.213	8.22	0.107	10.49	0.0826	10.963	0.0554
	20	7.68	0.138	7.57	0.275	9.53	0.0629	9.45	0.227	11.7	0.204	11.54	0.0826	13.85	0.0866	14.317	0.0415
	30	11.61	0.145	8.59	0.282	13.48	0.0479	10.45	0.204	15.65	0.183	12.55	0.108	17.8	0.0645	15.313	0.0375
	40	15.7	0.15	9.88	0.256	17.58	0.0702	11.75	0.208	19.76	0.202	13.85	0.108	21.92	0.0778	16.6	0.0289
	50	18.08	0.165	10.91	0.285	19.99	0.0688	12.76	0.205	22.15	0.204	14.86	0.12	24.3	0.0645	17.6	0.0354
	60	23.15	0.196	11.38	0.295	25.08	0.0854	13.25	0.187	27.21	0.175	15.35	0.0913	29.41	0.0688	18.137	0.0427
Stem diameter	**0**	1.975	0.0479	1.9	0.108	1.567	0.0568	1.613	0.0826	2.05	0.0866	2.038	0.208	2.225	0.16	2.075	0.0479
	10	2.43	0.0332	2.405	0.131	1.98	0.0913	2.03	0.119	2.58	0.0816	2.518	0.225	2.705	0.193	2.555	0.0479
	20	2.9	0.0645	2.795	0.111	2.475	0.0629	2.533	0.0898	3	0.0866	2.97	0.202	3.175	0.197	2.995	0.0854
	30	3.442	0.0545	3.032	0.119	3.002	0.0669	2.74	0.0712	3.51	0.05	3.172	0.22	3.685	0.193	3.257	0.0719
	40	3.88	0.05	3.072	0.113	3.468	0.0625	2.763	0.0794	3.98	0.0866	3.202	0.215	4.155	0.197	3.285	0.0731
	50	4.39	0.0289	3.12	0.105	3.98	0.0678	2.82	0.0881	4.49	0.05	3.24	0.218	4.692	0.184	3.313	0.0708
	60	4.88	0.05	3.11	0.121	4.49	0.0594	2.843	0.0826	5.018	0.0515	3.27	0.206	5.192	0.182	3.337	0.0778
Leaf number	**0**	6	0	6	0	6.5	0.5	6.5	0.5	7	0.577	7	0.57	7.5	0.5	7.5	0.5
	10	8	0	8	0	8.5	0.5	8.5	0.5	9	0.577	9	0.57	9.5	0.5	9.5	0.5
	20	8	0	8	0	8.5	0.5	8.5	0.5	9	0.577	9	0.57	9.5	0.5	9.5	0.5
	30	8	0	8	0	8.5	0.5	8.5	0.5	9	0.577	9	0.57	9.5	0.5	9.5	0.5
	40	10	0	8	0	10.5	0.5	8.5	0.5	11	0.577	9	0.57	11.5	0.5	9.5	0.5
	50	12	0	8	0	12.5	0.5	8.5	0.5	13	0.577	9	0.57	13.5	0.5	9.5	0.5
	60	16	0	8	0	16.5	0.5	8.5	0.5	17	0.577	9	0.57	17.5	0.5	9.5	0.5
Leaf area	**0**	7.75	0.73	7.675	0.18	8.348	1.527	8.24	0.679	11.157	1.63	10.82	0.724	14.793	0.754	15.415	0.382
	10	9.14	0.735	9.013	0.175	9.738	1.562	9.625	0.709	12.523	1.641	12.16	0.733	16.157	0.75	16.75	0.384

Vari.	DADB	*Ca*754				*Ca*J-19				*Ca*74110				*Ca*74112			
		ww		*ws*		*ww*		*ws*		*ww*		*ws*		*ww*		*ws*	
		M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
	20	10.73	0.73	10.305	0.199	11.328	1.527	10.945	0.735	14.113	1.616	13.48	0.731	17.748	0.771	18.045	0.398
	30	12.59	0.73	10.578	0.176	13.192	1.531	11.215	0.709	15.97	1.614	13.75	0.733	19.655	0.738	18.358	0.387
	40	14.51	0.73	10.668	0.176	15.11	1.529	11.323	0.715	17.892	1.615	13.85	0.724	21.532	0.76	18.445	0.382
	50	17.07	0.735	10.7	0.176	17.675	1.534	11.342	0.718	20.453	1.616	13.87	0.724	24.078	0.749	18.468	0.382
	60	19.85	0.697	10.717	0.176	20.435	1.568	11.363	0.718	23.233	1.616	13.89	0.724	26.868	0.771	18.488	0.381
RL	**60**	19.2	1.64	10.99	0.2	23.5	1.61	14.02	1.56	20.7	0.23	15.16	0.23	20.9	0.1	16.12	1.53
RN	**60**	47.3	1.64	26.51	0.22	51.5	1.64	30.73	1.41	48.5	0.22	35.52	0.12	51.4	0.13	40.14	1.55
RV	**60**	5.4	0.31	3.15	0.2	8.3	1.64	4.94	0.58	6.7	0.15	5.11	0.12	7.5	0.13	5.86	1.56

Appendix 8. Shoot visual symptoms of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110 and *Ca*74112, growing under (A) well-watered and drought-stressed conditions, (B) well-watered, and (C) drought-stressed (after 60 days of drought treatment). Pictures were taken using Sony Alpha A7RIV.



Appendix 9. Comparing the root growth differences of *C.arabica* genotypes growing under well-watered (ww) and drought stress (ws) conditions (after 60 days of drought treatment). Pictures were taken using Sony Alpha A7RIV.



Appendix 10. Comparing the biomass *of C. arabica* genotypes (A) growing under well-watered conditions and (B) growing under drought stress conditions (after 60 days after drought stress treatment). Pictures were taken using Sony Alpha A7RIV.

Appendix 11. Mean values and SD of relative water content (%), stem water potential (Ψw, −Mpa), net assimilation rate (A, µmol $m^{-2}s^{-1}$), stomatal conductance (Gs, mmol $m^{-2}s^{-1}$), and transpiration rate (E, mmol $m^{-2}s^{-1}$) of the four coffee (*Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112) genotypes.

Var.	DADB	*Ca*754				*Ca*J-19				*Ca*74110				*Ca*74112			
		ww		*ws*		*ww*		*ws*		*ww*		*ws*		*ww*		*ws*	
		M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
RWC	0	81.17	0.02	81.15	0.05	81.6	0.03	81.64	0.02	81.79	0.02	81.55	0.22	82.89	0.69	82.79	0.64
	10	81.22	0.02	72.83	0.03	81.72	0.23	72.34	0.02	81.91	0.6	76.1	0.26	83.02	0.53	77.59	0.64
	20	81.21	0.02	64.83	0.07	81.85	0.26	66.32	0.04	81.46	0.44	68.6	0.27	82.76	0.67	72.54	0.75
	30	81.16	0.03	57.55	0.05	81.57	0.18	59.3	0.18	81.7	0.39	62.25	0.6	82.86	0.37	67.29	0.64
	40	81.19	0.01	49.58	0.06	81.87	0.23	49.49	0.26	81.83	0.14	56.65	0.22	82.89	0.61	62.19	0.68
	50	81.45	0.25	40.31	0.12	81.3	0.31	41.54	0.1	81.92	0.35	48.55	0.47	82.32	0.47	54.84	0.75
	60	81.16	0.02	30.24	0.21	82.08	0.59	32.57	0.13	81.5	0.63	43.4	0.29	82.76	0.61	48.09	0.8
Stem water potential	0	-1.44	0.03	-1.44	0.02	-1.44	0.02	-1.43	0.06	-1.45	0.03	-1.44	0.02	-1.44	0.03	-1.45	0.02
	10	-1.49	0.01	-1.72	0.02	-1.41	0.04	-1.75	0.06	-1.4	0.04	-1.67	0.04	-1.45	0.04	-1.67	0.03
	20	-1.44	0.03	-2.11	0.03	-1.46	0.02	-2.14	0.06	-1.49	0.02	-1.91	0.02	-1.47	0.03	-1.91	0.02
	30	-1.48	0.03	-2.47	0.02	-1.43	0.03	-2.43	0.07	-1.41	0.04	-2.17	0.02	-1.43	0.02	-2.15	0.02
	40	-1.42	0.03	-2.69	0.03	-1.5	0.02	-2.6	0.07	-1.46	0.05	-2.31	0.02	-1.45	0.02	-2.27	0.02
	50	-1.44	0.03	-2.85	0.03	-1.45	0.03	-2.77	0.04	-1.44	0.02	-2.44	0.02	-1.47	0.02	-2.4	0.03
	60	-1.45	0.03	-3.11	0.02	-1.45	0.04	-3.02	0.07	-1.44	0.05	-2.64	0.02	-1.48	0.01	-2.56	0.02
Net assimilation rate	0	6.1	0.11	6.13	0.09	6.14	0.15	6.15	0.13	6.31	0.04	6.36	0.07	6.43	0.13	6.45	0.1
	10	6.16	0.07	5.28	0.09	5.83	0.12	5.44	0.09	6.37	0.06	5.63	0.06	6.44	0.13	5.91	0.16
	20	6.1	0.07	4.4	0.13	6.24	0.18	4.6	0.13	6.29	0.13	5.03	0.09	6.44	0.18	5.25	0.1
	30	6.68	0.38	3.51	0.09	6.41	0.3	3.81	0.18	6.72	0.33	4.28	0.07	6.87	0.28	4.69	0.11
	40	7	0.46	2.68	0.06	6.6	0.15	3.06	0.13	6.97	0.4	3.6	0.09	7.19	0.31	4.15	0.13
	50	7.28	0.19	1.79	0.09	6.68	0.25	2.3	0.14	7.04	0.45	3.02	0.1	7.34	0.3	3.55	0.1
	60	7.33	0.37	1	0.09	7.24	0.26	1.55	0.13	7.46	0.12	2.29	0.06	7.59	0.08	2.89	0.11

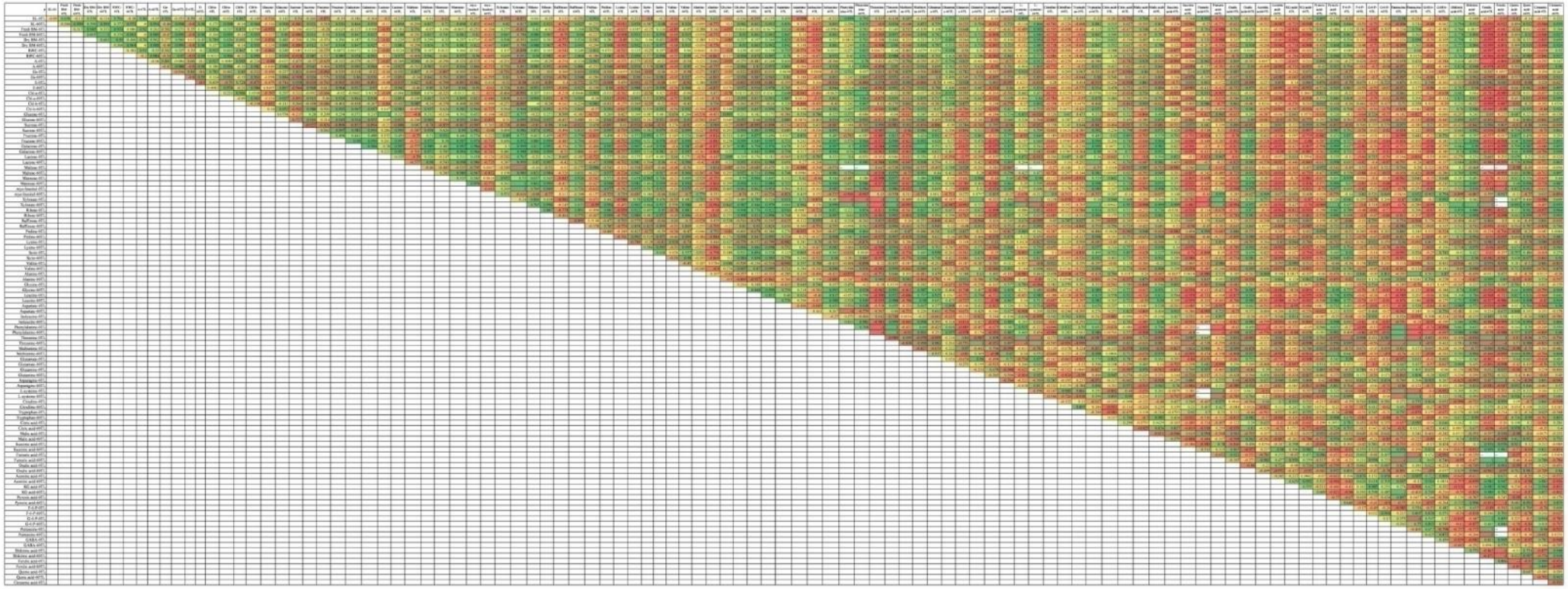
Var.	DADB	*Ca*754				*Ca*J-19				*Ca*74110				*Ca*74112			
		ww		*ws*		*ww*		*ws*		*ww*		*ws*		*ww*		*ws*	
		M	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**	**M**	**SD**
Stomatal conductance	**0**	97.25	0.75	98.99	0.82	97.39	0.55	99.49	0.8	97.38	0.85	99.09	0.97	97.63	0.75	102.09	1.37
	10	98.25	0.95	88	0.96	97.92	0.34	88.86	0.65	98.43	0.44	90.38	0.92	98.22	0.33	95.21	1.46
	20	99.25	0.75	77.27	0.87	99.24	0.63	78.88	0.71	99.52	0.69	81.22	1.32	98.52	0.21	88.18	1.42
	30	99.25	0.63	66.11	0.85	99.5	0.65	68.8	0.67	100.46	0.96	72.54	0.79	99.55	0.54	81.53	1.36
	40	99.5	0.96	54.11	0.9	99.87	0.43	57.81	0.84	100.47	1.1	64.26	0.95	101.45	1.46	73.54	1.53
	50	100.5	0.65	42.38	0.84	99.56	0.63	47.06	0.75	101.75	1.31	55.46	0.89	102.91	1.05	66.27	1.44
	60	101.75	0.48	30.28	0.86	101.89	1.52	36.84	0.71	102.63	0.99	46.51	0.89	104.69	0.89	60.25	1.38
Transpiration rate	**0**	3.15	0.05	3.3	0.04	3.325	0.075	3.375	0.047	3.575	0.047	3.6	0.04	3.625	0.049	3.75	0.045
	10	3.275	0.06	2.775	0.025	3.325	0.111	2.85	0.05	3.6	0.091	3.155	0.049	3.725	0.079	3.356	0.068
	20	3.325	0.062	2.295	0.047	3.35	0.126	2.37	0.028	3.6	0.141	2.715	0.049	3.725	0.075	2.965	0.075
	30	3.4	0.04	1.825	0.047	3.375	0.062	1.925	0.047	3.7	0.04	2.305	0.047	3.75	0.045	2.595	0.054
	40	3.4	0.091	1.355	0.047	3.425	0.103	1.44	0.05	3.825	0.047	1.895	0.079	4	0.242	2.245	0.064
	50	3.45	0.02	0.9	0.04	3.5	0.07	0.97	0.064	3.975	0.144	1.488	0.027	4.15	0.287	1.865	0.075
	60	3.575	0.075	0.415	0.047	3.525	0.085	0.54	0.028	4.025	0.229	1.11	0.04	4.3	0.324	1.565	0.075

Appendix 12. Logarisimicaly (Log_2) transformed fold change of metabolites of the coffee genotypes growing under ww and ws conditions, at 0 and 60 days after drought stress begins. *ww*, *ws* and *pw* refers to well-water, drought stress, and path way.

Category	Lists of metabolites	Fold changes															
		*Ca*754				*CaJ*-19				*Ca*74110				*Ca*74112			
		Log_2 (60/0 TL)		Log_2 (ws/ww)		Log_2 (60/0 TL)		Log_2 (ws/ww)		Log_2 (60/0 TL)		Log_2 (ws/ww)		Log_2 (60/0 TL)		Log_2 (ws/ww)	
		ww	ws	0TL	60TL	ww	ws	0TL	60TL	ww	ws	0TL	60TL	ww	ws	0TL	60TL
Sugars	Glucose	-0.58	1.17	0.42	2.17	-0.74	4.71	-0.32	5.13	0.00	8.65	0.58	9.24	0.00	9.04	0.00	9.04
	Sucrose	-0.02	0.63	-0.01	0.64	0.01	0.60	0.00	0.59	0.00	2.37	-0.02	2.36	-0.02	2.73	-0.03	2.72
	Fructose	-0.01	0.74	-0.01	0.74	0.00	0.86	-0.02	0.84	0.00	2.06	-0.01	2.05	0.00	2.23	0.00	2.23
	Galactose	-0.11	1.47	-0.11	1.47	-0.11	1.88	-0.11	1.88	0.00	4.69	0.10	4.79	0.00	5.24	0.09	5.32
	Lactose	0.00	0.71	-0.13	0.58	0.00	0.81	-0.22	0.58	-0.21	4.55	-0.10	4.66	-0.18	4.86	-0.18	4.86
	Maltose	0.00	4.41	0.32	4.73	0.00	4.71	0.42	5.13	0.00	6.94	0.32	7.26	0.58	7.16	0.32	6.90
	Mannose	0.02	0.09	0.01	0.08	0.01	0.19	-0.01	0.18	0.00	1.46	0.00	1.46	0.07	1.49	0.07	1.48
	myo-Inositol	0.00	-0.53	-0.01	-0.53	-0.01	-0.56	-0.01	-0.56	0.00	-0.58	0.00	-0.58	0.00	-0.56	0.00	-0.56
	Xylonate					-0.01	-0.64	0.00	-0.64	-0.01	2.11	-0.01	2.11	0.00	2.08	0.00	2.08
	Ribose	-0.04	0.93	-0.10	0.87	-0.09	1.07	-0.03	1.12	-0.02	3.13	0.00	3.14	0.08	3.34	0.06	3.32
	Raffinose	-0.07	0.07	0.00	0.14	-0.08	0.24	-0.08	0.24	-0.10	0.41	0.00	0.51	-0.13	1.84	-0.23	1.73
	Erythronate													-0.02	1.54	0.05	1.61
Amino Acids	Proline	0.03	0.46	-0.01	0.41	0.01	0.53	0.02	0.55	0.00	2.79	0.00	2.78	-0.01	3.06	-0.01	3.06
	Lysine	0.12	1.91	0.08	1.87	0.00	2.09	0.00	2.09	-0.08	3.74	0.00	3.83	0.00	4.50	-0.04	4.46
	Serine	0.01	1.19	0.03	1.21	-0.02	1.47	-0.01	1.48	-0.01	2.64	0.01	2.65	-0.02	3.28	0.07	3.37
	Valine	0.00	1.58	0.05	1.63	-0.01	1.70	-0.04	1.67	0.01	3.05	-0.01	3.03	-0.02	3.30	-0.04	3.28
	Alanine	0.01	-0.28	0.02	-0.27	-0.04	-0.07	0.01	-0.02	-0.02	-0.46	-0.03	-0.47	-0.01	-0.66	0.02	-0.63
	Glycine	0.01	0.01	0.06	0.05	0.04	0.36	-0.02	0.29	0.04	1.93	0.07	1.96	0.06	2.33	0.02	2.29
	Leucine	0.00	0.45	-0.03	0.42	-0.03	0.89	-0.05	0.88	0.02	3.29	-0.02	3.26	-0.07	3.78	0.02	3.87
	Aspartate	0.00	-0.60	-0.02	-0.62	0.00	-1.10	-0.01	-1.10	0.00	-0.85	-0.01	-0.85	0.00	-0.67	-0.01	-0.67
	Isoleucine	0.01	0.20	0.03	0.22	-0.02	0.23	-0.01	0.23	0.04	3.47	0.02	3.45	-0.03	3.61	-0.04	3.60
	Phenylalanine	0.09	0.11	0.03	0.05					-0.02	1.58	0.03	1.63	0.12	1.46	0.03	1.37
	Threonine	0.00	0.98	0.03	1.01	0.04	0.88	0.06	0.90					-0.04	3.82	-0.08	3.78
	Methionine	0.00	-0.29	-0.06	-0.35	0.08	-0.20	0.08	-0.20	0.04	4.39	-0.13	4.22	-0.20	3.98	-0.16	4.02
	Glutamate	0.00	-2.90	-0.01	-2.90	0.02	-2.60	-0.02	-2.64	0.00	-2.70	-0.01	-2.71	0.04	-2.69	0.03	-2.70
	Glutamine	0.01	-2.09	0.00	-2.10	0.01	-2.15	0.03	-2.13	-0.03	-2.45	-0.02	-2.44	0.01	-0.74	-0.01	-0.77
	Ornithine					0.00	-0.62	-0.06	-0.68	-0.12	0.40	-0.18	0.33				
	Asparagine	0.03	-1.37	0.07	-1.34	0.03	-1.75	0.03	-1.75	0.09	-0.87	-0.06	-1.03	-0.06	-0.88	-0.06	-0.88
	L-Cysteine	0.00	0.81	-0.32	0.49					0.00	1.00	0.00	1.00	-0.42	5.18	-0.42	5.18
	Citrulline	3.32	2.50	0.58	-0.23	-0.32	-0.32	-0.32	-0.32	0.00	1.42	-0.42	1.00	-0.17	-0.81	-0.36	-1.00
	Tryptophan	0.22	-0.81	0.22	-0.81	0.00	-1.22	0.22	-1.00	0.00	7.38	0.58	7.97	0.00	8.68	-0.58	8.09

Category	Lists of metabolites	Fold changes															
		*Ca*754				*CaJ*-19				*Ca*74110				*Ca*74112			
		Log_2 (60/0 TL)		Log_2 (ws/ww)		Log_2 (60/0 TL)		Log_2 (ws/ww)		Log_2 (60/0 TL)		Log_2 (ws/ww)		Log_2 (60/0 TL)		Log_2 (ws/ww)	
		ww	ws	0TL	60TL	ww	ws	0TL	60TL	ww	ws	0TL	60TL	ww	ws	0TL	60TL
TCA cycle pw	Citric acid	-0.01	-0.87	-0.01	-0.87	0.00	-1.88	-0.01	-1.89	0.00	-0.68	-0.03	-0.71	0.00	-0.21	0.01	-0.20
	Malic acid	0.01	1.75	0.00	1.74	0.00	1.55	0.01	1.56	0.00	2.37	0.00	2.37	0.00	2.32	0.00	2.32
	Succinic acid	0.01	-0.01	0.00	-0.01	0.00	0.05	-0.04	0.01	0.00	-0.63	0.00	-0.63	-0.01	-1.19	0.00	-1.18
	Fumaric acid	0.00	3.32	-0.32	3.00	0.32	-0.74	0.32	-0.74					-0.32	0.00	-0.32	0.00
	Oxalic acid	0.02	0.39	0.01	0.39	0.01	0.26	0.05	0.30	0.00	1.21	0.00	1.21	0.00	1.49	-0.01	1.48
	Isocitric acid					0.00	-0.42	0.42	0.00	-0.42	-1.58	-0.42	-1.58				
	Aconitic acid	0.01	-0.63	0.00	-0.63	0.00	-0.83	0.00	-0.83	0.00	-1.42	0.00	-1.42	0.00	-1.81	0.00	-1.81
	α-Ketoglutaric acid	0.09	-0.09	0.09	-0.09	0.00	0.00	-0.17	-0.17	0.00	-0.83	-0.09	-0.92	-0.18	-2.32	-0.18	-2.32
Glycolysis pw	Pyruvic acid	0.03	-0.51	0.06	-0.48	0.19	-0.39	0.35	-0.23	0.04	3.75	0.09	3.79	0.04	3.72	0.00	3.68
	Fructose-6-phosphate	0.09	-0.35	-0.10	-0.54	-0.08	-0.39	-0.16	-0.47	-0.08	-1.85	-0.08	-1.85	0.19	-1.09	0.28	-1.00
	Glucose-6-phosphate	0.13	-0.78	0.19	-0.72	-0.05	-0.61	-0.16	-0.72	0.06	-3.00	0.00	-3.06	-0.13	-1.74	-0.20	-1.81
GABA shut pw	SSA	0.00	0.00	0.00	0.00									0.00	-0.26	0.26	0.00
	Putrescine					0.19	-1.00	0.19	-1.00	0.17	-1.42	0.00	-1.58	-0.36	-0.81	-0.36	-0.81
	γ-Aminobutyric acid	0.03	-1.02	0.03	-1.01	0.03	-1.33	0.02	-1.34	0.00	-1.46	-0.01	-1.46	0.00	-1.06	0.00	-1.06
Shikimic pw	Shikimic acid	-0.03	0.97	-0.04	0.97	0.00	1.07	0.00	1.06	0.00	1.54	0.00	1.54	0.00	1.55	0.00	1.55
	Chlorogenic acid									-0.04	-0.52	-0.04	-0.52	0.00	-1.04	0.08	-0.96
	Ferulic acid	0.42	1.17	0.42	1.17					0.00	0.00	0.00	0.00	-0.42	0.00	-0.42	0.00
	Quinic acid	0.00	-0.39	-0.01	-0.40	-0.09	-0.36	-0.06	-0.34	0.01	0.02	0.00	0.01	-0.02	0.03	0.01	0.06
	Cinnamic acid	0.26	-1.58	0.26	-1.58	-0.26	-1.00	0.00	-0.74	0.22	0.00	0.00	-0.22	-0.22	0.00	-0.22	0.00

Appendix 13. Pearson correlation analysis and heat-map of growth, physiology, and metabolites of coffee genotypes of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well-water and drought stress conditions, both at 0-TL and 60-TL periods.



Appendix 14. At $r > 0.8$, summary statistics of metabolites network analysis of coffee genotypes of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, under well-water and drought stress conditions, both at 0-TL and 60-TL periods.

At r > 0.8, average summary statistics of metabolites network analysis	**Relatively tolerant coffee genotypes (*Ca*74110, and *Ca*74112)**	**Sensitive coffee genotypes (*Ca*754 and *Ca*J-19)**
Number of nodes	87	80
Number of Edges	1250	1411
Average number of neighbours	29.762	36.293
Network diameter	10	3
Network radius	5	2
Characterstics path length	4.268	1.094
Clustering coefficient	0.924	0.991
Network density	0.359	0.907
Network heterogenity	0.385	0.211
Network centralization	0.126	0.071

Appendix 15. Heat-map and PCA score value representing the total metabolite's up- and down-accumulation of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, after 60 days after drought begins. Red, the lowest value; green, highest value; and, in between colors (yellow, orange, light green) represent the medium value.

Metabolites	**PC 1**	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**
Glucose	11.96	0.66	-0.34	1.57	-0.26	0.06	-0.24	0.06
Maltose	9.79	3.46	-0.10	-0.08	0.13	0.44	0.15	0.08
Tryptophan	8.08	-4.73	-0.72	-0.72	-0.29	0.32	-0.14	0.01
Galactose	5.47	0.12	0.17	0.06	0.17	-0.17	-0.08	0.03
Lactose	4.50	-1.14	-0.20	-0.24	0.01	-0.39	-0.01	-0.04
Lysine	4.42	0.97	0.34	0.00	-0.05	-0.06	0.05	0.00
Methionine	3.23	-1.91	-0.57	-0.12	0.08	-0.14	0.18	-0.16
Leucine	2.99	-0.49	0.16	0.20	0.10	-0.12	0.04	-0.01
Valine	2.94	1.07	0.09	-0.04	-0.03	-0.09	0.01	0.00
Ribose	2.71	0.17	-0.06	0.08	0.16	-0.12	-0.01	-0.05
Isoleucine	2.70	-0.98	-0.23	-0.12	0.06	-0.06	0.05	0.04
Pyruvic acid	2.70	-1.82	-0.50	-0.01	0.18	0.25	0.19	-0.11
Serine	2.60	0.70	0.38	0.14	0.07	-0.04	0.02	0.04
L-Cysteine	2.17	-0.97	2.67	-0.39	-0.13	-0.16	-0.19	-0.13

Metabolites	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
Proline	2.04	-0.35	-0.05	0.02	0.08	-0.04	0.04	-0.02
Malic acid	1.86	1.50	-0.07	-0.13	0.03	-0.04	-0.02	-0.03
Threonine	1.80	0.27	1.55	-0.11	-0.06	0.02	0.01	0.08
Sucrose	1.60	0.01	0.05	-0.07	0.05	-0.06	0.02	-0.01
Fructose	1.17	0.45	-0.03	0.07	0.05	-0.06	-0.01	-0.03
Glycine	0.84	-0.37	-0.02	0.18	0.05	0.02	-0.02	0.05
Xylonate	0.57	-0.78	-0.33	-0.60	0.08	-0.05	0.04	0.17
Shikimic acid	0.49	1.06	-0.07	0.12	0.06	-0.03	-0.04	-0.08
Erythronate	-0.02	0.01	-0.02	0.01	0.08	0.01	0.02	0.06
Phenylalanine	-0.02	-0.09	-0.40	0.14	0.09	0.02	0.00	-0.01
Mannose	-0.05	-0.06	-0.20	0.14	0.12	-0.01	0.01	0.02
Oxalic acid	-0.11	0.26	0.02	0.00	0.05	0.03	0.01	-0.02
Raffinose	-0.48	0.08	0.67	0.14	-0.16	-0.07	-0.10	-0.07
Fumaric acid	-0.76	1.83	-0.93	-2.56	0.07	0.01	-0.16	-0.08
Ornithine	-0.91	-0.36	0.67	-0.53	-0.02	-0.14	0.10	0.19
Succinate Semialdehyde	-1.13	0.39	-1.11	0.27	0.29	0.02	0.03	0.01
Ferulic acid	-1.76	1.38	-0.07	-0.51	-0.55	0.12	0.02	0.09
Isocitric acid	-1.97	0.39	2.15	-0.05	0.00	0.13	0.44	-0.12
Quinic acid	-2.20	-0.01	-0.17	0.23	0.06	-0.02	-0.06	-0.01
Citrulline	-2.21	0.30	-1.62	0.16	-0.58	-0.30	0.34	0.08
Cinnamic acid	-2.76	-1.01	-0.17	0.76	-0.24	0.13	0.06	-0.10
Alanine	-2.85	0.59	-0.24	0.40	0.05	0.05	-0.02	-0.09
Chlorogenic acid	-2.94	1.18	-0.38	0.21	0.08	0.04	-0.01	-0.02
myo-Inositol	-3.09	0.07	-0.18	0.21	0.06	0.04	-0.06	-0.03
Succinic acid	-3.25	1.00	-0.47	0.28	0.03	0.02	-0.09	-0.08
Citric acid	-3.40	-1.10	0.03	-0.49	0.10	0.00	-0.02	0.19
Aspartate	-3.51	-0.25	-0.10	-0.09	0.07	0.02	-0.06	0.04
Putrescine	-3.87	0.55	0.42	-0.64	-0.24	0.17	-0.07	-0.10
Asparagine	-4.11	-1.05	-0.22	-0.04	0.01	0.06	0.04	0.05
Fructose-6-phosphate	-4.19	0.49	0.46	0.40	0.26	-0.09	-0.08	0.14
α-Ketoglutaric acid	-4.27	1.25	-1.05	0.27	-0.18	-0.10	-0.09	-0.13
γ-Aminobutyric acid	-4.33	-0.43	0.03	0.06	0.05	0.09	-0.03	0.05
Aconitic acid	-4.56	0.39	-0.43	0.18	0.06	0.07	-0.08	-0.04
Glutamine	-5.24	-1.61	0.80	0.28	0.07	0.11	-0.02	0.07
Glucose-6-phosphate	-5.63	0.66	0.76	0.41	-0.30	0.05	-0.14	0.07
Glutamate	-7.01	-1.79	-0.39	0.58	0.14	0.10	-0.05	-0.02

Appendix 16. Heat-map and PCA score value representing the drought tolerance capacity of the four coffee genotypes, *Ca*754, *Ca*J-19, *Ca*74110, and *Ca*74112, after 60 days after drought begins. Red, the lowest value; green, highest value; and, in between colors (yellow, orange, light green) represent the medium value.

Genotype and treatment	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
*Ca*74110-*ws/ww*-60TL	0.977	-0.077	-0.199	-0.009	0.002	-0.001	0.001	-0.001
*Ca*74112-*ws/ww*-60TL	0.972	-0.151	0.180	-0.008	-0.001	0.000	0.000	0.002
*Ca*J-19-*ws/ww*-60TL	0.803	0.538	0.041	0.254	-0.003	0.002	0.000	-0.010
*Ca*754-*ws/ww*-60TL	0.679	0.673	0.041	-0.290	-0.002	-0.002	-0.003	0.009
*Ca*74110-*ws/ww*-0TL	0.594	-0.144	-0.153	0.124	-0.015	0.500	-0.574	0.095
*Ca*754-*ws/ww*-0TL	0.142	0.103	-0.316	0.382	-0.664	0.305	0.307	0.309
*Ca*J-19-*ws/ww*-0TL	0.099	-0.002	0.184	-0.501	0.251	0.680	0.329	-0.268
*Ca*74112-*ws/ww*-0TL	0.023	0.339	-0.066	0.409	0.786	0.061	0.124	0.275

Appendix 17. Laboratory and lath-house studies, including seed washing, testing, treatment and pre-germination data measurements, as well as germination trial experiments, at Addis Ababa University. Pictures were taken using Sony Alpha A7RIV.

Appendix 18. Preliminary activities, including soil-compost-sand preparation, sterilization, weighing, and pot filling, before transplanting the germinated seedlings, at Addis Ababa University. Pictures were taken using Sony Alpha A7RIV.

Appendix 19. Screening chlorosis and leaf rust infected coffee genotypes, and the respective pest management activities in the green-house studies, at Addis Ababa University. Pictures were taken using Sony Alpha A7RIV.

Appendix 20. Transplanting of the coffee genotypes, trial growth and physiological experiments, seedlings managements, sample extraction for biomass, pigment, cell membrane stability and metabolite studies, and freezing leaf and root samples in Liquid Nitrogen before storing at -80^{O}C, at Addis Ababa University. Pictures were taken using Sony Alpha A7RIV.

Appendix 21. Laboratory biochemical and molecular studies, at Nicolause Copernicus University in Torun, Poland. Pictures were taken using Sony Alpha A7RIV.

Appendix 22. Articles published during the Ph.D. study period.

Article one: a*griculture*, Volume 13, Issue 9, 1754, August 2023, 3.6 *if*, 3.6 citescore.

agriculture

MDPI

Article

Unraveling Drought Tolerance and Sensitivity in Coffee Genotypes: Insights from Seed Traits, Germination, and Growth-Physiological Responses

Habtamu Chekol [1,*], Yimegnu Bezuayehu [1], Bikila Warkineh [1], Tesfaye Shimber [2], Agnieszka Mierek-Adamska [3], Grażyna B. Dąbrowska [3] and Asfaw Degu [1]

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Abstract: The coffee plant is highly susceptible to drought, and different genotypes exhibit varying degrees of tolerance to low soil moisture. The goal of this work was to explore the interrelation between seed traits and germination events, growth patterns, and physiological responses of coffee genotypes, aiming to identify significant associations that may facilitate the selection of coffee genotypes exhibiting enhanced drought tolerance and yield potential. Two consecutive experiments were conducted to examine the impact of these factors. In the first experiment, germination performance was examined for three groups of coffee genotypes: relatively tolerant (*Ca*74140, *Ca*74112, and *Ca*74110), moderately sensitive (*Ca*74158, *Ca*74165, and *Ca*J-21), and sensitive (*Ca*754, *Ca*J-19, and *Ca*Geisha). The subsequent experiment focused on the growth and physiological responses of two relatively tolerant (*Ca*74110 and *Ca*74112) and two sensitive (*Ca*J-19 and *Ca*754) genotypes under drought stress condition. The relatively tolerant genotypes showed quicker and more complete germination compared to other groups. This was associated with higher moisture content, higher seed surface area to volume ratio, and higher coefficient of velocity of germination, coefficient of variation of germination time, and germination index. Additionally, the relatively tolerant genotypes showed higher seedling vigor. The results of the second experiment demonstrated superior growth performance in relative tolerant genotypes compared to the sensitive groups. Young coffee plants belonging to relatively tolerant genotypes exhibited higher growth performance than the sensitive genotypes, with a net assimilation rate strongly correlated to relative water content, leaf number, stomatal conductance, and chlorophyll-a. In addition, a strong correlation was exhibited between the growth of young coffee plants and the surface area to volume ratio of the seeds, as well as the germination percentage. The seedling vigor index showed a strong correlation with net assimilation rate, chlorophyll content, seedling growth, and cell membrane stability. Furthermore, principal component analysis illustrated distinct clustering of genotypes based on their germination and growth-physiological performance. Overall, the findings of this study suggest that seed traits, germination, and post-germination events are integral factors in determining drought tolerance and sensitivity, as well as the growth and physiological responses of adult coffee plants.

Keywords: Arabica coffee; drought; genotype; seed; germination; moisture content; seedling vigor; gas exchange; cell membrane stability

Citation: Chekol, H.; Bezuayehu, Y.; Warkineh, B.; Shimber, T.; Mierek-Adamska, A.; Dąbrowska, G.B.; Degu, A. Unraveling Drought Tolerance and Sensitivity in Coffee Genotypes: Insights from Seed Traits, Germination, and Growth-Physiological Responses. *Agriculture* **2023**, *13*, 1754. https://doi.org/10.3390/agriculture13091754

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1. Introduction

Coffea arabica L. is the most widely cultivated commercial species, accounting for over 70% of the world's coffee production [1,2]. It is believed that the south and southwest of

Article two: *plants*, Volume 13, Issue 6, 828, March 2024, 4.5 *if*, 5.4 citescore.

plants

MDPI

Article

Drought Stress Responses in Arabica Coffee Genotypes: Physiological and Metabolic Insights

Habtamu Chekol [1], Bikila Warkineh [1], Tesfaye Shimber [2], Agnieszka Mierek-Adamska [3], Grażyna B. Dąbrowska [3] and Asfaw Degu [1,*]

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Abstract: Understanding the impact of drought stress on Arabica coffee physiology and metabolism is essential in the pursuit of developing drought-resistant varieties. In this study, we explored the physiological and metabolite changes in coffee genotypes exhibiting varying degrees of tolerance to drought—namely, the relatively tolerant *Ca*74110 and *Ca*74112, and the sensitive *Ca*754 and *Ca*J-19 genotypes—under well-watered conditions and during terminal drought stress periods at two time points (0 and 60 days following the onset of stress). The metabolite profiling uncovered significant associations between the growth and the physiological characteristics of coffee genotypes with distinct drought tolerance behaviors. Initially, no marked differences were observed among the genotypes or treatments. However, at the 60-day post-drought onset time point, notably higher shoot growth, biomass, CO_2 assimilation, pigments, and various physiological parameters were evident, particularly in the relatively tolerant genotypes. The metabolite profiling revealed elevations in glucose, maltose, amino acids, and organic acids, and decreases in other metabolites. These alterations were more pronounced in the drought-tolerant genotypes, indicating a correlation between enhanced compatible solutes and energy-associated metabolites crucial for drought tolerance mechanisms. This research introduces GC-MS-based metabolome profiling to the study of Ethiopian coffee, shedding light on its intricate responses to drought stress and paving the way for the potential development of drought-resistant coffee seedlings in intensified agro-ecological zones.

Keywords: drought stress; *Coffea arabica*; growth; gas exchanges; metabolites; network analysis

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1. Introduction

Coffee stands as a vital global agricultural commodity, trailing only behind oil in importance. Its production in tropical and subtropical regions sustains millions of livelihoods [1,2]. *Coffea arabica* L. accounts for over 70% of the world's coffee production and is famed for its excellence [3]. Brazil leads in Arabica coffee production at 44%, with Ethiopia contributing 5% [4]. Ethiopia, a top Arabica coffee producer in Africa, exports about 471,000 tons yearly, yielding 0.71 tons per hectare [5–7]. The looming specter of global climate change, however, threatens *C. arabica* cultivation with water scarcity and drought. This poses significant challenges to coffee cultivation, disrupting suitable regions, yield, and quality, and inviting pests and diseases, causing economic losses [8,9].

Plants instinctively adjust their catabolic and anabolic systems during drought, altering metabolic pathways to protect against damage [10,11]. This adaptation's mechanism hinges on the species, genotype, and stress intensity [12,13]. Metabolic adjustment involves accumulating compatible solutes, influencing pathways like sugar synthesis, photosynthesis, and more [14]. Certain metabolites increase during drought stress, like proline, serine, valine, and betaine,

DECLARATION

I, the undersigned, declare that this is my original work, has not been presented for a Ph.D. degree in any other University and that all sources of materials used and data generated for the study have been fully acknowledged. I cede the copyright of the dissertation in favor of Addis Ababa University.

Habtamu Chekol Fantahun

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