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CLIMATE CHANGE PARTNERSHIP ACTIONS FOR SUSTAINABLE FUTURE AND RESTORING LIFE ON EARTH

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PREFACE

There is a growing concern on the adverse impacts of climate on biodiversity. This phenomenon is greatly manifested in form of shifting weather patterns threatening global food security, health and species existence. Humanity is at the receiving end of the consequences of climate change hence there is a need to step up actions on all fronts- overtime, everywhere all at once.

This calls for collaboration, partnership and networking to strengthening synergy among relevant stakeholders in a bid to tackling climate change menace. This forms the basis for the theme of this year world Environmental conservation conference: **CLIMATE CHANGE PARTNERSHIP ACTIONS FOR SUSTAINABLE FUTURE AND RESTORING LIFE ON EARTH.** The theme is conceived with a view to create an interface for information sharing and offer opportunities for participants to refine their commitments and pledges in the quest to achieving Sustainability in the face of climate change.

This year World Environmental Conservation Conference is memorable in the sense that it received overwhelming funding from the host - West African Science Service on Climate Change and Adapted Land use). WASCAL is posed to provide information and knowledge at the local, national and regional level to cope with the adverse impacts of climate change. Thus, this conference will offer opportunities for participants to learn from good practices demonstrated and showcase by WASCAL during the course of the conference. It will also strengthen staff-student exchange and provide prospect for Doctorate Research Doctoral Research in West Africa Climate System Programme (DRP WACS) – WASCAL among others.

Special appreciation goes to the management of The Federal University of Technology, Akure the host institution, National Park Service and African Regional Center for Space Science and Technology Education-English (ARCSSTE-E) that co-host this conference. We equally acknowledge other private, individual and corporate organizations that have contributed towards the success recorded in this event.

All the submitted articles were subjected to strict double blind peer-review process by the reviewers that are experts in the area of the particular submitted manuscript. The accepted manuscripts are published in WECC 2023 proceedings and also available for download on the organization website (www.necorng.org).

The accepted manuscripts fall within the underlisted subthemes:

- Climate change adaptation strategies in Agriculture, Forestry and Other Land Use (AFOLU)
- Climate smart city and architectural landscape design
- Retrofitting and decarbonization in tourism and hospitality industry
- Indigenous knowledge and local innovation in climate change adaptation
- Climate risk management, health, safety and hygiene
- Carbon credit-offset marketing/circular economy
- ICT development in environmental conservation (image processing and acquisition, computer vision, graphics, speed, interface technology, HMD devices, GIS: Body Tracking, AI and IOT, VRT, IVE).

We commend our keynote speaker Prof. Douda Kone Director Capacity Building Department, WASCAL Headquarter, Ghana and other guest speakers Prof. Babatunde Rabiu, Director General, Chief Executive Office, African Regional Centre for Space Science and Technology Education-English (ARCSSTE-E) and Dr. Goni I. M., Conservator General National Park Service.

It is hoped that researchers, students and policy makers will find the papers in this book very useful. Even though all the papers were reviewed and edited, the content and option expressed remain essentially that of the authors and not necessarily that of Netlink Environmental Conservation Organization.

Dr. Oladeji S. O.

President Netlink Environmental Conservation Organization

Convener World Environmental Conservation Conference

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EFFECTS OF DROUGHT AND REHYDRATION ON THE GROWTH AND BIOCHEMICAL ATTRIBUTES OF CITRUS PROVENANCES: IMPLICATIONS FOR SEEDLING MORTALITY AND SURVIVAL

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ABSTRACT

Experiments were conducted at the screenhouse of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure Nigeria, to determine effects of drought and rehydration cycles on growth and physiological attributes and biochemical constituents of citrus provenances. Seedlings were raised from Citrus paradisi (Grapefruit), Citrus aurantium (Bitter orange), Citrus tangelo (Tangelo),Citrus limon (Lemon, Citrus sinesis (Sweet orange) and Citrus maxima (Shaddock).The seedlings were initially exposed to watering regimes at 100, 70 and 50 % filed capacity watering for ten (10) months under screenhouse condition. Thereafter, the seedlings were exposed for three (3) months (12 weeks) under wet-dry cycles consisted of well watering (100 % FC), wet-dry (watering at fortnightly intervals) and dry down treatment (eight weeks of drought for which rewatering commenced when symptoms of seedling mortality was noticed.Data were collected on the growth (plant height, number of leaves and root (tap root length, root fresh and dry weights, number of roots and total root length), physiological data: Chlorophyll contents and biochemical Attributes: Proline, soluble sugar content, phenolic content, membrane lipid peroxidation and antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Gluthathione (GSH) and non-structural carbohydrates (NSCs). The imposed drought and rehydration cycles affected the growth, physiological and biochemical attributes of seedlings of citrus provenances.Total and tap root length, root biomass, plant height and number of branches were affected by drought and rehydration episodes in citrus. The provenances differed in their responses to drought and rehydration episodes with respect to osmolytes concentrations, antioxidative and enzymatic activities of catalase (CAT), superoxide dismutase (SOD), Guaiacol peroxidase (GPx) and non-structural carbohydrates (NSCs). Treatments induced significant changes in enzymatic activities of catalase (CAT),Guaiacol peroxidase (GPx) and non-structural carbohydrates (NSCs). Under non-drought (adequate watering) condition, total phenol,chlorophyll, sugars (fructose, sucrose and glucose) concentrations were higher while at rooztzone moisture stress) conditions, leaf contents of DPH, superoxide dismutase (SOD) and proline were higher while CAT and NSC were relatively lower compared with non-drought stress situation. Under limited water availability condition, fortnightly watering intervals is recommended for citrus provenances evaluated while dry down and rewatering high soil moisture limitation. The study confirmed the drought susceptibility of citrus to wet-dry cycles involving various periods of drought and rehydration. The recuperative potentials (recovery of physiological function) of citrus provenance following relief from drought episodes differed and is related to changes in morphological, physiological attributes and enzymatic antioxidant activities. This information can guide the choice of citrus species as rootstock in vegetative propagation.

Keywords: *Fruit trees, climate adaptation, rootzone moisture, physiology, photochemistry, growth, recovery*

INTRODUCTION

Citrus (*Citrus species*) are widely cultivated in both tropical and subtropical climates (Raga, 2016). Citrus has been recognized as a group of fruit trees of great importance for global agricultural trade based on thelarge number of citrus-producing countries worldwide (Ziogas *et al*., 2021). The species is particularly are rich in Vitamin C (ascorbic acid) and folic acid and are good source of fiber. In Nigeria, an estimated 3 million hectares of land, about 930,000 tons of citrus fruits are produced annually (Mbah *et al*. in 2018).The health benefits of citrus are enormous: the tree produces fruits of important to human nutrition (vitamins, minerals and bioactive phytochemicals, soluble fibre,). The fruit is low in cholesterol and calories, lowers risk of kidney stones by raising level of citrate in human urine, protects against esophageal, stomach, breast and pancreatic cancers. The flavonoid contents acts as antioxidants (flavonoid called naringin) and can benefit heart health by improving cholesterol levels and lowering blood pressure. The flavonoids found in citrus fruits have anti-inflammatory capabilities which help protect human against the chain of events that causes the nervous system to deteriorate. Kerri-Ann Jennings (2017), reported that citrus fruits and juices may help boost brain function and protect the brain from neurodegenerative disorders.

Climate change-enhanced increases in duration, intensity and frequency of drought, dry spells and soil moisture deficits coupled with rising temperatures being experienced worldwide (Agele, 2021), causes impairment of growth and physiological functions in plants(Chaudhry *et al.,* 2022, Ogunwole *et al*. 2023). Water stress negatively affects growth and impairs cell metabolism, affecting the overall tree growth and produced fruit quality (Carr, 2012, Olayemi *et al.,* 2022). Plants under drought stress, demonstrate significant reduction of growth and cellular metabolic processes, with a concomitant reduction in crop yield and fruit quality (Sah *et al*., 2016, Ogunwole *et al.,* 2023a). Plants cope with drought stress conditions through physiological, biochemical, anatomical, and morphological modifications (Chaudhry et al., 2022, Ogunwole *et al.,* 2023b). Plant responses to drought aim to reduce loss of cellular turgidity (cell water loss), protecting intracellular structures and molecules, and repairing damage caused by free radicals (Kaur and Asthir, 2017, Regina, 2018). Cell dehydration tolerance mechanisms are also characterized by accumulation of osmo-protectants, antioxidants, and reactive oxygen species (ROS) scavengers, as well as the biosynthesis of cell-protecting proteins, such as HSPs and hydrophilins (Olayemi *et al.,* 2022, Ogunwole *et al.,* 2023), Plants also respond to drought via production of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, as well as metabolites such as ascorbate and glutathione, to reduce ROS toxicity.

In plants, abiotic stress factors especially water stress triggers production of reactive oxygen species (ROS) leading to an increase in membrane lipid peroxidation measured as malondialdehyde (MDA) content (Moller et al., 2007, Ogunwole et al., 2023c). Under water deficit stress, remarkable increase in MDA content of in seedlings of *Eucalyptus globulus* (Correia et al., 2014), *Jathropha curcas* (Arcoverde *et al.,* 2011) and sunflower (*Helianthus* species) (Cechin et al.,2010) are reported. Reactive oxygen species production correlates favorably with drought resistance of citrus (Seday *et al*., 2014), this is to overcome oxidative stress, plants develop enzymatic and non-enzymatic antioxidant defense mechanisms to scavenge ROS (Smirnoff, 1993, Aranjuelo et al., 2010*)*. Drought stress leads to oxidative stress due to stomatal closure (Lei et al., 2006, Ozkur et al., 2009) and formation of reactive oxygen species (ROS) in chloroplasts and mitochondria (Fu and Huang, 2001, Akram et al., 2016, Abdelaal et al., 2020).

Deployment of protection mechanisms against moisture deficit stress constitutes an important tolerance strategy under water stress (Chaves et al., 2009). In curtailing the impacts of oxidative stress, plants increase the activity of antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Guaiacol Peroxidase (GPx) and Malondialdehyde (MDA) under drought conditions to manage the accumulation of ROS (Abdelaal et al., 2020). Comparably higher activities of antioxidant enzymes have been reported in drought tolerant plant species than in the sensitive (Bor et al., 2003). In addition, osmotic adjustment is apparently a reliable mechanism that confers an ecological advantage on large percentage of trees by maintaining metabolic activity under water deficit stress (Aranjuelo et al., 2010, Akram et al., 2016). Thus, drought tolerance is effected through the accumulation of osmo-protective substances such as proline, ascorbic acid, total soluble sugar, late embryogenesis abundant proteins, phenols, and flavonoids. Increase in the content of these biochemical constituents under water deficit stress is a common strategy to maintain favourable osmotic potential (Aranjuelo et al., 2010), which play essential role in improving plant immunity for enhanced species tolerance against oxidative stress (Akram et al., 2016; Ogunwole et al., 2023b).

Climate change, variability and extremity of weather manifest have increased warming and drought with consequences on growth, vigour and physiological functions in plants. Knowledge is inadequate on the effects of drought and episodes of soil moisture deficits on citrus species widely cultivated in Nigeria Knowledge gap also exists on physiological and biochemical mechanisms of tolerance of moisture deficits in native and exotic citrus species in the nursery. It is imperative to evaluate the responses of citrus provenances widely grown in fruit tree orchard in Nigeria and identify suite of growth, physiology and biochemical attributes which confer drought tolerance on these horticultural species. It is hypothesized that, citrus provenances selected differ in their responses to soil moisture status and in the suite of attributes which confer drought tolerance on them at the seedling stage. The aim of this study thus, iattributes as well as biochemical constituents of citrus species; anddeploy measured variables to rank tolerance to soil moisture deficits by citrus species

MATERIALS AND METHODS

The experiment was conducted at the screen house of the Crop, Soil and Pest Management Department, the Federal University of Technology, Akure (FUTA), Nigeria.

Analyses of physiological and biochemical attributes were carried out in the Crop, Soil and Pest Management, Biology and Biochemistry Laboratories, FUTA.

Planting Materials

The citrus species evaluated were:

1. *Citrus paradisi* (Grapefruit)
2. *Citrus aurantium (*Bitter orange)
3. *Citrus tangelo (*Tangelo)
4. *Citrus limon (*Lemon)
5. *Citrus sinesis* (Sweet orange)
6. *Citrus maxima (Shaddock)*

The experiment was a 6 x 3 factorial combinations of citrus and watering regimes arranged in Completely Randomized Design (CRD) with 8 replications.

Treatment

The seedlings were initially exposed to watering regimes at 100, 70 and 50 % filed capacity watering for ten (10) months under screenhouse condition. Thereafter, the seedlings were exposed for three (3) months (12 weeks) under wet-dry cycles consisted of well watering (100 % FC), wet-dry (watering at fortnightly intervals) and dry down treatment (eight weeks of drought for which rewatering commenced when symptoms of seedling mortality was noticed.

Plant Measurements

Growth data: Plant height, number of leaves and root (tap root length, root fresh and dry weights, number of roots and total root length.

Analysis of physiological and biochemical attributes of citrus

Morphological Attributessuch as plant height, stem girth, number of leaves, root length and root: Fresh weight of root biomass was recorded using accurate weighing balance.

Physiological and biochemical attributes: Chlorophyll content, proline, soluble sugar content, phenolic content, membrane lipid peroxidation and antioxidant enzymes (Superoxide dismutase (SOD), Catalase (CAT), Gluthathione (GSH) were determined

Non Enzymatic Antioxidants Accumulation: Leaf proline and Soluble Sugars levels respectively follows Bates *et al.*, (1973) and Anthrone method of Irigoyen *et al.*, (1992) procedures, leaf Phenolic Acids follows Folin Ciocalteau method (Ainsworth and Gillespie, 2007) and Aluminium Chloride colorimetric assay- (Zhishen *et al.*, 1999).

Enzymatic antioxidants activities: Estimation of lipid peroxidation especially, level of Malondialdehyde (MDA) lipid peroxidation followed the procedures of Hodges *et al.*, (1999). The photochemical repression of nitrobluetetrazolium (NBT) by superoxide dismutase (SOD) was monitored. Using method of Giannopolitis and Ries (1977]. Catalase activity (CAT, EC. 1.11.1.6) was determined according to the method described by Aebi and Lester (1984) and expressed in terms of mM of H_2O_2 per minute per gram of FW (mMol min^{-1} g^{-1} FW) taking $\varepsilon = 40$ mM^{-1} cm^{-1} as extinction coefficient of H_2O_2 at 240 nm. Guaiacol peroxidase (GPx) activity was determined via enzyme extraction of uaiacol and the oxidation of guaiacol to tetraguaiacol using spectrophotometer from where change in absorbance was recorded at 15 s interval for 2 min. The GPx activity was estimated as 0.01 unit increase in absorbance due to formation of tetraguaiacol (extinction coefficient $\varepsilon = 26.6$ $mM^{-1}cm^{-1}$) and presented as mM tetraguaiacol formed min^{-1} mg^{-1} FW (Rao *et al.*, 1996).

Membrane Stability was determined via: Level of lipid peroxidation in the cell membranes . malondialdehyde (MDA) content was determined citrus leaves following the procedure of Hodges *et al.*, (1999). Superoxide dismutase (SOD) activity – (Giannopolitis and Ries, 1977). Peroxidase and catalase activity respectively follows Aebi and Lester, (1984) and Rao *et al., (*1996) procedures.

General methodologies for determining biochemical constituents and enzymatic antioxidants activities:The contents of proline, soluble sugar (TSS), ascorbic acid (Asc) and phenolic acid (TPC) on fresh weight basis were estimated according to the procedures of Bates et al. (1973), Irigoyen et al., (1992), AOAC (2000) and Ainsworth and Gillespie (2007) respectively. Peroxidation of membranes lipid measured as malondialdehyde (MDA) content was determined by following the methods of Hodges et al. (1999). For assessment of the activities of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPx), the procedures of Giannopolitis and Ries (1977), Aebi and Lester (1984) and Chance and Maehly (1955) were respectively followed.

Statistical Analysis

All experiments were conducted in three replicates and the data obtained were subjected to two way ANOVA using Minitab statistical software. Treatment Means was separated using the Tukey's Honest Significant Difference at $P < 0.05$.

RESULTS

Effects of watering strategies on growth and physiological attributes of citrus

From data collected on citrus growth variables (height, number of leaves and root (tap root length, root fresh and dry weights, number of roots and total root length) showed that these variables were influenced by watering treatments imposed. The leaf chlorophyll contents of citrus species had close values while significant differences were found for watering treatments. Decrease in the chlorophyll content of leaves was observed with decreasing water application (well watering, short term wet-dry cycles and down) (Table 1). Watering at dry down enhanced proline, SOD, DPPH, Phenol ABTS, GPx, CAT contents followed by short term wet-dry while its was lowest for well watered condition which however enhanced leaf chlorophyll concentration and non structural carbohydrate (NSC).

The responses of citrus provenances to watering regime differed.

Although lemon had the highest content of chlorophyll under 100% FC watering regime and bitter orange however has the highest content of chlorophyll. For wet dry cycles, lemon had highest content of chlorophyll and dry down, chlorophyll contents of citrus leaves declined

compared with values under well watering and wet dry cycle. Citrus species differently accumulated proline in response to watering regimes. Grape and sweet orange, under well watering and short term wet dry treatments, close values proline were obtained, and values were lower than under dry down (highest proline content). But for bitter orange and tangelo and lemon, sweet orange, increases in proline concentration in leaves were found with decreasing water application. Grapefruit under both 100 and 75% FC has the lowest content of proline while sweet orange under 75% FC has the highest content of proline followed by sweet orange under 50% FC.
Effects of watering regimes on glutathione (GSH) levels of citruswith decreases in soil moisture status (well watering, short term wet dry and dry down treatments) It was observed that there were no significant differences in the content of GSH among citrus species based on watering regimes. Grapefruit, sweet orange and tangelo haveclose values of GSH under well watering and wet dry treatments and highest content of GSH under dry down. Watering strategies affected glutathione peroxidase (GPx) activity of citrus, Results showed that grapefruit, bitter orange and tangelo respectively had close values (similar response patterns) across under watering regimes. Bitter orange has close values for GPx under dry down and wet dry cycle, lemon has its highest GPx under dry down and lowest under well watering condition while sweet orange has its highest GPx under dry down treatment.

The effects of watering regimes on superoxide dismutase (SOD) activity of citrus showed the existence of non-significant differences for SOD content of citrus leaves for well watering and wet dry cycles.Grapefruit has its highest SOD content under dry down, bitter orange had lowest for well watering condition and in tangelo, grape, lemon and sweet orange had close values of SOD content under both well watering and wet dry.

Catalase activity (CAT) of citrus species differed under the various watering strategies. Grape has its highest CAT activity under dry down and similar for bitter orange and tangelo. For lemon, highest CAT activity was observed under dry down and wet dry cycle and lowest under well watered condition. Among the species, sweet orange has highest content of CAT activity and under dry down while lowest was grape.

In summary, among the provenances, the largest number of roots is found for grape under wet dry compared with well watering and dry down. For grape, tangelo and bitter orange plants grown under dry down condition, longest tap roots were produced while lemon and sweet orange had longest roots under wet dry treatment. Tangelo and sweet orange had largest number of roots under well watering and wet dry cycles while lemon has the greatest number of roots under dry down and wet dry for tangelo. For lemon, proline, SOD and DPPH were highest while these variables had lowest values for grape in addition to lowest glucose and NSC. In contrast, grape had highest chlorophyll concentrations and ABTS in leaves. Tangelo has high contents of ABTS and among provenances, the highest sucrose and NSC contents. Sweet orange produced highest proline ,SOD, DPPH, Phenol, CAT, fructose and glucose in its leaves. In case of shaddock, the leaf contents of proline and SOD DPPH Phenol, ABTS, CAT were high in addition to high contents of fructose and NSC. Bitter orange has high contents of CAT, glucose and NSC but lowest for sucrose.

DISCUSSION

The reduction in the vigour of growth of citrus plants under short term wet-dry cycles and dry down condition can be attributed to limited water availability in the rootzone This observation is consistent with the report of Elemike *et al*. (2019) that decrease in soil water negatively affects plant growth. The differences in growth attributes of citrus provenances for growth variables may be explained genotypic capacity for growth.

The high capacity of citrus to tolerate unfavourable climatic conditions was reported by Vinod (2012). Soil moisture deficit tolerance capacity differed among provenances, for variables such as number of leaves, height and root biomass under the watering strategies. Nicholas (1998) reported that water stress reduced the biomass of fibrous root in avocado cultivars. Lemon has the highest root biomass under the various watering strategies considered. In particular, heaviest root biomass was obtained for lemon under dry down treatment compared with other species. The huge investment in root development would have conferred advantage via soil moisture extraction similar to observations of Ogunwole et al. (2023) on urban forest tree species response to severe soil moisture deficit stress.

Results showed that chlorophyll content of citrus leaves was lowest under dry down condition. Kumar *et al*. (2011) reported that plant leaves undergo decrease in chlorophyll content under drought. Decreased chlorophyll contents may results on deterioration of chloroplast structure due to oxidative stress under soil moisture deficit stress. The well watering treatment produced the highest chlorophyll contents among species followed by short term wet-dry cycles and lowest under dry down condition. According to Ashraf and Harris (2013), the oxidative damage of chloroplast lipids and proteins or the degradation of the pigment protein complexes by chlorophyllase enzyme could cause chlorophyll loss. This loss of chlorophyll can also resulted on limitations in light capturing system (PS-I), energy transfer or antenna complex accessories (Nikolaeva *et al*., 2010, The biochemical traits of citrus species were affected by watering strategies adopted. One of the early reactions of plant to water stress is that it adjusts the intercellular osmotic potential through the accumulation of organic compatible osmolytes such as sugars and proline (Girardi *et al*. 2017, Olayemi et al., 2022). The reported increases in proline accumulation in plants in response to water deficit is useful as a possible drought injury sensor (Vendruscolo *et al*., 2007, Girardi *et al*. 2017) which as an important strategy by plants to avoid detrimental effect of water stress. Thus, proline is involved in the tolerance mechanisms against oxidative stress in plants. Due to limited water around the roots, proline accumulates in cells to obtain suitable condition for taking up water (Tatar and Gevrek, 2008, Gonçalves *et al*., 2016, Ogunwole at al., 2023). Proline is an amino acids, its use as biochemical marker of water stress in citrus is suggested by Zaher-Ara *et al*. (2016). This study showed that there was continuous increase in the proline accumulated by the species with decreasing water regime especially for grapefruit, sweet and bitter orange and tangelo (Shao *et al*., 2008). Lemon had outstanding proline accumulation and can explain its best drought performanceunder dry down and short term wet dry cycles. However, stress-induced proline content is reversible (Sharma and Verslues, 2010). Kishor and Sreenivasulu, 2014) noted that proline is remobilized after periods of rehydration (post drought hydration), proline is degraded to release energy and nitrogen for the cell growth. The decreases in contents of leaf proline with decreasing water application (decreasing trends were: well watering, short term wet dry and dry down) may stem from the accumulation of proline far in excess of the demands of protein synthesis under increasing soil moisture deficit stress (Kaur and Asthir, 2015, Ogunwole et al., 2023) Environmental stresses affect antioxidant enzymes activities in plants. Higher levels of antioxidant enzymes activities was observed with decreasing water application (resultant increasing soil moisture deficit stress form well watering, resultant increasing soil moisture deficit stress (dry down and short term wet dry). Antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD) and guaiacol peroxidase (GPx) increased with increasing soil moisture deficit stress (short term wet dry and dry down treatments based on results from this study). Lemon showed high content of CAT, SOD and GPx under stress (50% FC) which may have contributed to its relatively higher soil moisture deficit stress tolerance compared with other citrus species. Although sweet orange has the highest content of SOD and CAT under dry down condition, compared with other species, it also has the high content of SOD and CAT as well as GPx under short term wet dry treatment. This responses may denote that SOD and CAT are not suitable markers (indicators) of effect of rootzone ,moisture deficit stress in sweet orange. Sanchez- Rodriguez *et al*. (2011) obtained differences in CAT activity of tomato in responses to varying status of soil moisture. Most of citrus species evaluated produce increases in SOD, CAT and GPx content with rootzone moisture deficits (short term wet dry cycle) compared with dry down treatment for which these antioxidants may be suitable as markers for water stress in the citrus. Thus, the degree of rootzone moisture deficit stress examined resultantly from the various watering strategies (well watering, short term wet dry cycle) was detrimental to citrus seedlings for citrus provenances exhibited different degrees of tolerance to rootzone moisture deficit stress. It also appeared that the citrus species evaluated deployed the antioxidants (GPx specifically) for moisture deficit stress amelioration/alleviation.. The activities of CAT is species specific, sweet orange expressed the highest CAT activity under dry down condition followed by lemon.

The contents of chlorophyll, proline, phenols and soluble sugar contents culminated to enhanced MDA lipid peroxidation. Thus, accumulation of high chlorophyll, phenols as well as high activities of catalase and guaiacol peroxidase may account for high leaf turgidity and least malondialdehyde lipid peroxidation in citrus.High proline and soluble sugar accumulation in suboptimal watering regimes may be attributed to proline effect on turgor potential maintenance. This observation is consistent with reports for black poplars (Regier *et al.,* 2009), mulberry (Reddy *et al.,* 2004), eucalyptus (White *et al.,* 2000), oaks (Cotrozzi *et al.*,2016) and Conocarpus, Salix and Acacia(Rasheed *et al.,* 2021). Under moisture deficit condition, phenolic compounds acts as antioxidants [Khan *et al.*,2015]; neutralizing free radicals (ROS) by quenching singlet and triplet oxygen and/or decomposing peroxides developed in the chloroplasts (Osawa, 1994). Such responses may result in maintenance of photosynthetic apparatus and membrane cell integrity. The increased levels of phenols in citrus seedlings may serve in activating defense mechanisms via up-regulating phenolics-synthesizing enzymes, such as phenylalanine ammonia-lyase found for *Eucalyptus globulus*(Gondor *et al.*,2016) , *Syzygium cumini*(Zafar *et al.*,2021) in suboptimal watering condition.Increased activities of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOx) at suboptimal soil moisture status i confirms the role of antioxidant enzymes system in protection of plant cellular organelles from toxicity effects of reactive oxygen species (ROS). Under drought, plants initiate defense mechanisms through activation of SOD which functions as both precursor for highly reactive oxygen derivatives - peroxynitrite and hydroxyl radical (Halliwell and Gutteridge, 1999) and as ROS scavenger through dismutation of 0_2 to H_2O_2 in suboptimal watering condition (Mittler2002). Thus, up-regulation of the activity of CAT and GPOx would bring about decreases in b cellular H_2O_2 for drought improve tolerance of these species to suboptimal watering.

Similar enhancement of the activities of SOD and CAT was reported for *Acacia modesta* (Rasheed *et al.,* 2021), peach (Haider *et al.*,2018), grapes (Zhang *et al.*,2017) and olive plants (Ahmed *et al.*,2009). Chlorophyll pigments loss in citrus species under suboptimal watering treatment supports the possible involvement of antioxidants system in preserving the PSII functional integrity in water stressed plants. Oxidative burst injures the cellular organelles, protein structure, causes nucleic acid fragmentation and impairs other physiological processes (Lei *et al.*,2006) leading to increased malondialdehyde (MDA) lipid peroxidation. In plants, the damage of membrane lipids increased MDA content of citrus seedlings (Tschaplinski *et al.*,2019). In this study, suboptimal watering induced ROS generation in citrus seedlings. The high peroxidation of membrane lipids may pose threat to survival and establishment of UFTS under suboptimal watering condition. These results corroborate the reports on pistachio(Khoyerdi *et al.*, 2016).

CONCLUSIONS

Growing citrus under variable status brought about in its growth attributes, physiological biochemical attributes and drought susceptibility. The study affirmed differences in drought susceptibility of seedlings of citrus provenances at short term wet dry cycles and dry down conditions. These treatments elicited changes in growth and physiological attributes and biochemical constituents.Citrus seedling growth attributes significantly reduced under deficit water application (short term wet dry and dry down treatments) which had profound effects on accumulation of osmolytes, osmoprotectants and enzymatic activities of citrus seedlings. In particular, dry down treatment induced remarkable accumulation of osmolytes and osmoprotectants and enzymatic activities. Response of growth and physiological traits of citrus to wet-dry cycles, it is established that adequate watering produced optimal growth, development and vigour of citrus seedlings which may enhance field establishment and reduce mortality. The short term wet dry and dry down treatments enhanced the accumulation of proline, malondialdehyde (MDA) and activities of enzymatic antioxidants in citrus seedlings compare with 100% FC watering. dry down treatment enhanced the activities of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPOx) while short term wet dry treatment produced up regulation of the activities of CAT and GPOx.Differences were observed among the citrus species for growth traits when subjected to watering regimes treatment. The values of growth traits of plant height, root length and biomass of citrus v were lower under soil moisture deficits: dry down and short term wet dry compared with the well watering situations. It appeared that lemon was more tolerant to soil water deficit, followed by bitter orange, grape and sweet orange. Tangelo displayed the least tolerance of rootzone moisture deficit. In case where there is water is inadequate/limited in availability, short term wet dry watering strategy can be adopted for citrus, especially lemon, bitter orange, grapefruit and sweet orange while dry down should be avoided for citrus seedlings especially in the nursery, The study provided information and improved understanding of the relevance of morphological, physiological and biochemical traits to soil moisture deficit stress tolerance in citrus species.Findings from the study can serve as a guide in the choice of citrus species for rootstock in vegetative propagation. In case where there is water is inadequate/limited in availability, 75% FC can be employed for citrus, especially lemon, bitter orange, grapefruit and sweet orange while dry down should be avoided for citrus seedlings especially in the nursery Findings from the study can serve as a guide in the choice of citrus species for rootstock in vegetative propagation, and for horticulturists and policymakers for making pro-active plans for climate change adaptation and mitigation.

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Table 1. Effects of watering strategy on some growth attributes of citrus provenances

Citrus species	Watering regime	Tap root Length (cm)	Total root length (cm)	Fresh root weight (g)	Plant height (cm)
Lemon	Well watered	33	53	11.2	23.7
	Wet-dry	68	78	7.8	30.2
	Dry down	42	64	17.4	33.3
Tangelo	Well watered	21	87	13	23.4
	Wet-dry	39	131	17	28.2
	Dry down	63	112	11	20.5
Sweet orange	Well watered	31	73	12.5	28.3
	Wet-dry	62	86	17.7	23.4
	Dry down	74	81	9.5	25.1
Shaddock	Well watered	20,2	43	9.3	32.4
	Wet-dry	27.4	61	13.2	28.5
	Dry down	33.1	72	17.4	22,7
Grape	Well watered	51.3	94.3	13.4	28.3
	Wet-dry	64,1	121	16.7	24.1
	Dry down	78.5	135	18.2	20.3
Bitter orange	Well watered	53.3	76.3	12.4	32.2
	Wet-dry	61,5	84.3	18.1	43.1
	Dry down	67.1	91.3	20.3	37.4
	Watering	*	*	*	*
	Provenance	*	*	*	*
	Wr* Pr	*	*	*	ns

* (significance at P > 0.05), ns (non significant @ P > 0.05)

Table 2. Effects of watering strategy on some biochemical (osmolytes and enzymatic antioxidant activities) of citrus provenances

Citrus species	Watering regime	Proline (mg/g)	SOD (%)	Chloro Phyll (mg/g)	DPPH (%)	Phenol (mg/g)	ABTS (mol/g)	GPX µ/g	CAT (μM^{-1} mg-1)	Water soluble carbohydrates (mg/g)			NSC (mg/g)
										Fructose	**fructose**	**glucose**	
Lemon	Well watered	0.0055	29.804	36.32	73.284	19.69	0.022062	162.86128	10.50871	150.75	149.63	151.58	1.373767
	Wet-dry	0.0045	41.177	28.27	76.898	18.28	0.022142	196.05277	17.00895	144.29	142.19	145.15	1.395564
	Dry down	0.0039	49.021	35.83	81.102	19.33	0.022023	182.43574	17.603078	135.67	134.67	136.43	1.139447
Tangelo	Well watered	0.0016	25.49	28.16	69.46	20.14	0.025533	201.37191	14.223	183.71	182.32	184.71	0.801591
	Wet-dry	0.00078	17.65	21.22	71.13	21.07	0.025494	182.86128	12.99109	202.46	200.96	203.59	0.744919
	Dry down	0.00156	22.17	27.29	75.69	20.23	0.025733	180.30809	17.87793	200.37	198.89	201.49	0.753637
Sweet orange	Well watered	0,0273	37.26	48.57	80.38	19.83	0.025294	222.22298	9.938964	197.83	196.36	198.92	1.249523
	Wet-dry	0.0226	29.42	41,71	83.32	21.71	0.025254	206.69106	12.31245	201.31	199.82	202.43	1.192851
	Dry down	0.0289	37.26	46.92	88.38	20.81	0.024696	215.62723	10.78345	198.05	196.59	199.16	1.11765
Shaddock	Well watered	0.027	37.26	48.57	85.38	19.69	0.025294	222.22298	9.938964	203.85	202.35	204.96	1.159065
	Wet-dry	0.0226	29.42	45.71	85.32	17.12	0.025374	220.30809	12.69129	191.79	190.38	192.87	1.173233
	Dry down	0,0289	37.26	46.93	87.38	18.73	0.025294	220.09532	10.50871	185.36	164.13	166.28	0.977058
Grape	Well watered	0.014	52.94	25.31	62.17	11.66	0.018592	226.05277	10.92628	203.16	201.66	204.29	1.481663
	Wet-dry	0.0148	45.10	26.53	60.93	10.12	0.018392	216.05277	9.482034	180.89	179.56	181.91	1.613536
	Dry down	0.0136	45.11	22.45	58.51	8.94	0.018791	214.77617	10.57608	188.54	187.16	189.61	1.374857
Bitter orange	Well watered	0.0172	68.63	39.99	70.32	19.21	0.025533	190.52085	5.481289	202.69	201.21	203.83	1.304016
	Wet-dry	0.0180	70.34	38.73	75,93	20.71	0.025374	184.5634	8.307749	204.11	204.33	204.99	1.173233
	Dry down	0.0164	76.47	46.11	62.17	18.65	0.025573	191.79745	10.57608	206.43	205.75	206.39	1.242984
Watering		*	*	*	*	*	*	*	*	*	*	*	*
Provenance				ns						ns	ns	ns	
Wr* Pr													

*(significance at P > 0.05), ns (non significant @ P > 0.05)

Table 3. Effects of watering strategy on osmolytes and enzymatic antioxidant activities) of citrus

Watering strategy	Proline (mg/g)	SOD (%)	Chlorophyll (mg/g)	DPPH (%)	Phenol (mg/g)	GPX μ/g	CAT (μM^{-1} mg-1)	Fructose (mg/g)	Sucrose (mg/g)	Glucose (mg/g)	NSC (mg/g)
Well watering	0.01382	32.7662	37.9583	75.6033	17.9132	14.5532	185.675	143.574	142.16	144.38	1.30292
Wet dry	0.01466	33.0352	33.0352	75.6225	17.9737	17.9737	192.888	154.556	153.06	155.43	1.1122
Dry down	0.01545	44.765	32.2211	83.1866	18.3091	18.2168	197.631	173.946	172.65	174.91	0.89531
LSD (0.05)	*	*	*	*	*	*	*	*	*	*	*

*(significance at P > 0.05), ns (non significant @ P > 0.05)

Table 4. Effects of citrus provenance on osmolytes and enzymatic antioxidant activities

Citrus species	Proline	SOD	Chlorophyll	DPPH	Phenol	ABTS	GPX	CAT	fructose	sucrose	glucose	NSC
Lemon	0.0141	0,0046	40.213	33.373	33.013	77.09	19.1034	0.22	180.44	11.706	161.57	152.16
Tangello	0.0135	0.0013	30.774	29.556	25.556	72.09	20.4804	0.0255	188.18	15.697	195.51	194.06
Sweet orange	0.0257	0.0257	34.646	45.733	45.733	84.02	20.7833	0.0253	214.84	11.011	199.06	197.59
Shaddock	0.0261	0.0261	34.646	47.075	47.071	86.02	18.5133	0.0253	220.87	11.046	193.66	185.62
Grape	0.0141	0.0141	37.716	41.763	24.763	60.53	15.2432	0.0185	218.96	10.328	190.86	189.46
Bitter orange	0.0172	0.0152	35.953	35.614	41.612	69.47	19.5233	0.0254	188.96	8.8372	204.43	203.76
LSD (0.05)	*	*	ns									

*(significance at P > 0.05), ns (non significant @ P > 0.05)