Response of *Cyclopia Subternata* to Watering Frequency: Stomatal Conductance, Proline, and Relative Water Content

Mary-Jane S. Mahlare[1], Muinat N. Lewu [2], Francis B. Lewu [1*] and Cecilia Bester[2]

***Abstract*—** *Cyclopia*, commonly referred to as honeybush, is an indigenous tea plant native to the Eastern and Western Cape provinces of South Africa and is known for its sweet taste and honey-like aroma. The tea is famous for its antioxidants and can be used in value-added products such as cosmetics and other food ingredients. It is estimated that there are 23 *Cyclopia* species in South Africa, but only six are used commercially. Studies on abiotic stresses in honeybush are limited and this study helped to investigate the response of the species to water stress mechanisms, which is of utmost importance for the development of drought resistant lines for this highly sought-after tea plant. A pot experiment was conducted on a Stellenbosch granite soil in which *Cyclopia subternata* plants were subjected to three different watering frequencies (thrice, twice and once a week). More frequent watering (control) showed highest percentage of plant growth than plants subjected to other watering treatments in all the three growth parameters investigated. Higher proline concentrations and lower relative water content were observed in the water stressed plants (watering twice and once a week). Stomatal conductance was generally lower in stressed plants and highest in well-watered plants. The drop in stomatal conductance in the stressed plants is due to the induction of stomatal closure which is a coping mechanism to aid survival by reducing transpiration rate.

***Keywords*—** *Cyclopia*, stomatal conductance, proline content, relative water content, water stress

I. INTRODUCTION

*I*T is said that tea is the second most consumed beverage in the world after water. The type of tea (oolong, green, black, and herbal) usually depends on the post-harvest treatment [1]. Three different types of tea are grown in South Africa: Rooibos (Aspalathus linearis), bush (Athrixia phylicoides) and honeybush (*Cyclopia* species). While bush tea is still relatively unknown as a commercial product, honeybush has gained popularity, with rooibos being the best known and most established [2]-[4]. The market for honeybush is expected to grow due to the health benefits derived from it. Polyphenols, the antioxidants in honeybush tea, have beneficial effects on human health [5], [1]. Traditionally, honeybush has been used to treat disorders such as heartburn, ulcers, colic in infants, chronic tonsillitis, lung infections, nausea and mucus build-up in the respiratory tract or body cavities [3], [6]-[8]. There are 23 species of *Cyclopia* in the Cape Floristic Region of South Africa, of which only six are used commercially, among the six is *Cyclopia subternata*. About 82% of honeybush is still harvested in the wild [9]-[11]. These species have a very limited range and rare habitat requirements.

Abiotic stressors such as drought are undoubtedly one of the most limiting factors for plant growth [12], [13]. Plant growth is mostly limited by the unavailability of water and climate change is expected to increase the extent of water stress on agricultural soils [14], [15]. Plants accumulate proline and carbohydrates as a coping mechanism for water stress [8]. Though some authors question the direct relationship between proline accumulation and stress adaptation [16], others concluded that proline as a multifunctional molecule can serve as an osmolyte and radical scavenger by responding to a variety of abiotic and biotic stressors, or as a source of energy for regrowth by degrading in response to stress [17].

A plant's response to water stress is largely determined by the regulation of stomatal conductance. Water scarcity leads to stomatal closure, which is one of the first responses to water shortage [18]. The relative water content (RWC) of a plant can be used to determine how well or poorly it absorbs water and the extent of stress [19], [20]. RWC is defined as "the percentage of water present in the leaf as a fraction of the total volumetric water that the leaf can hold at full turgor" [21]. Under drought conditions, RWC is said to be a more accurate indicator of water status than any other metric of water potential. Leaf water supply and transpiration rate are closely linked and can give a good indication of the balance between these two variables [22]. Therefore, farmers need to understand how water use affects plant growth to maximize their productivity [23].

South Africa's arid climate, characterized by hot, dry conditions and low relative humidity, resulting in uneven distribution of rainfall and high evapotranspiration often leads to water stress [24]. There is very little research on how honeybush responds to drought stress in the Mediterranean climate of South Africa, where there is persistent drought during summer periods [25], [5], [8]. Therefore, the aim of

Mary-Jane S. Mahlare1 and Francis B. Lewu1 are with the Department of Agriculture, Faculty of Applied Sciences, Cape Peninsula University of Technology, Private Bag X8, Wellington 7654, South Africa

Muinat N. Lewu 2and Cecilia Bester2 are with ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

.

https://doi.org/10.17758/IICBE4.C1122203

this study was to evaluate the physiological and morphological responses of *C. subternata* to different watering frequencies.

II. MATERIALS AND METHODS

Study site and experimental design

A glasshouse pot study was conducted at the Agricultural Research Council (ARC), Infruitec, Stellenbosch (-33.925920º, 18.874259°) South Africa to assess the effect of water deficit stress on *C. subternata*. The experiment was conducted for 112 days (from mid-May until early September 2021). The physical and chemical properties of the soil medium are presented in Table I. The trial was a randomized block design (RBD) with three irrigation treatments [watering thrice (control), twice and once a week] and eight replicates.

Soil collection and planting

The soil (Stellenbosch granite) was collected from the ARC Nietvoorbij Research Farm and sieved to remove plant debris and larger fragments, in preparation for transplanting. Soil samples were collected for physicochemical analysis. Each pot was filled with14 kg of soil in a 30 cm (top diameter) plastic pot. The soil in each pot was irrigated to pot capacity (PC) before transplanting nine months old *C. subternata* seedlings, one plant per pot.

Watering treatments

All plants were watered uniformly with 300 ml of water for the first 81 days after transplanting (DAT) to ensure uniformity and strong root growth, before treatment application. Thereafter, *C. subternata* plants were subjected to three irrigation treatments (from early August to early September 2021) until the study was terminated at 112 DAT. The watering treatments were irrigating thrice (3 days/week), twice (2 days/week) and once (1 day/week). 300 ml of water was applied per pot, at every irrigation.

III. DATA COLLECTION

Growth parameters

Growth parameters were measured weekly from the second week of August until the second week of September 2021, when the study was terminated. Plant height was measured with a tape measure; stem diameter was measured using a digital Vernier caliper while stem circumference was calculated from stem diameter values using the following formula: $C = \pi d$,

where π = 3.14 and d= diameter (1)

Stomatal conductance

Stomatal conductance was measured with an SC-1 leaf porometer (Decagon Devices, Pullman, USA). The equipment measures the rate of passage of water vapour or carbon dioxide (CO2) through the leaf stomata. Measurements were taken on the abaxial (bottom) side of the leaf at mid-day, which corresponds with the peak period of the environmental factors.

Relative water content (RWC)

RWC of *C. subternata* leaves was determined weekly using an improved version of the method of Sade et al. [26]. Leaf samples (five uppermost leaves per plant) were collected at midday (12:00 noon), cut in half, and immediately placed in pre-weighed, labeled glass vials to minimize loss of moisture or vapour. Samples were kept on ice during collection and rapidly transported to the laboratory for the determination of RWC. Fresh weight (FW) per sample was determined using a sensitive weighing balance. To each vial, 2 mL of distilled water was added and kept in a dark cabinet at room temperature for 4 hours to rehydrate. The turgid leaf samples were removed from the vials and lightly blotted with a paper towel to remove excess water. The blotted leaves were weighed to determine the turgid weight (TW) and then dried in an oven at 70°C for 48 hours. The dried samples were later weighed to determine the dry weight (DW). RWC was determined using the formula below:

$$RWC = \frac{FW - DW}{TW - DW} X\ 100 \tag{2}$$

Estimation of proline content using the colorimetric method

Proline content of *C. subternata* was determined using the modified method of Abraham et al. [27]. Leaf samples were collected at weekly intervals during the study period. Extraction was done on fresh frozen leaves by crushing 0.1 g leaf sample in 0.5 mL of 3% sulfosalicylic acid (w/v). Using a 10 mm quartz glass cuvette, the absorbance was read at 520 nm on the UV-visible spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences, Waltham MA, USA). Proline concentrations of the *C. subternata* leaf samples were determined from the proline standard curve. Proline content was calculated using the formula below:

$$mmoles\ per\ g\ tissue = \frac{mg\frac{proline}{mL} x\ mL\ toluene}{115.5} x\ \frac{5}{g\ sample} \tag{3}$$

Where 115.5 = molecular mass of proline.

Shoots yield

Shoot yield was determined by cutting the shoot just above the soil surface, using a pruning shear. The harvested shoots were placed in a labeled paper bag and weighed with a sensitive scale, followed by oven-drying at 70^0 C for 24 hours. The dried weights were also recorded.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA, 2000), utilizing time as a sub-plot component for each observation time (sampling/week) separately [28]. The Shapiro-Wilk test was used to test for deviation from normality [29]. To compare treatment means, Fisher's least

https://doi.org/10.17758/IICBE4.C1122203

significant difference was determined at the 5% level [30]. For all tests, a probability level of 5% was considered significant.

IV. RESULTS AND DISCUSSION

Physical and chemical properties of the soil

Table I presents the physical and chemical properties of the soil medium used in this trial. The soil was classified as coarse sandy loam with a slightly acidic pH of 5.3 and a stone volume of 0.22 %. The nutrients found in the soil were within normal ranges for plant growth.

Growth parameters

As shown in Table II, in general, the three watering frequencies had no significant effect ($p > 0.05$) on plant height, although significant effects on stem diameter and stem circumference were observed during the first two weeks of the observation period. However, the plants watered thrice weekly, having significantly lower stem diameter and stem circumference quickly caught up with the less frequently watered plants from the third week of treatment application with no significant difference among all treatments. This could be attributed to better growth in the well-watered treatments. In the last week of observation, irrigation once a week had the poorest performance in terms of plant height, stem diameter and circumference. The results obtained on the growth parameters of *C. subternata* in this study agree with the findings of Tshikhudo et al. [31] who reported that the growth parameters of bush tea increased with increasing rainfall. As a thermophilic evergreen woody species, tea plant is very sensitive to low temperatures which affects its productivity. In response to low temperatures, the plants adapt to the cold by going into dormancy to survive potentially damaging weather conditions [32]. Therefore, the reason why there was generally no significant effect on the growth of *C. subternata* in this study, especially in plant height, may be due to the fact that this trial was carried out in winter. Hence, the winter season and the short duration of the experiment might have contributed to the findings of this study. The percentage change in plant height, stem diameter and stem circumference as influenced by watering frequency (Table III) highlight the average mean difference in growth parameters from the start of the treatments to the termination of the trial. The well-watered plants (control) showed the highest percentage growth in all three parameters, while plants under water deficit stress (twice and once a week) showed the least growth. As the irrigation frequency increased, the percentage growth also increased and vice-versa.

Shoot yield

The results of the effect of irrigation treatments on the shoot of *C. subternata* are shown in Table IV. No significant difference ($p > 0.05$) was found in the fresh and dry shoot yield in all treatments. These data agree with [33] who found that there was no significant effect on the leaf biomass of Aloe vera under short-term water deficit. However, the findings of this study contrasts Eziz et al. [34] who reported that water availability generally increases plant growth and biomass, and vice versa. According to Zhao et al. [35], a significant decrease in plant biomass was observed in Brassica napus grown under water deficit conditions. The reason for the results in this study may be influenced by the accumulation of proline as a defense mechanism against drought. The short observation period might also have contributed to the results.

Relative water content (RWC)

Fig. 1A shows differentiation of RWC in *C. subternata* leaves when plants were subjected to different water deficit stress over a period of five weeks. From the second week onwards, watering frequency had a significant effect ($p < 0.05$) on the treatments. Well-watered plants consistently had higher RWC than plants watered once or twice a week, with irrigation once a week having the least RWC. Other studies on olives, potatoes and *C. subternata* came to similar conclusions as this study, where water-stressed plants had the lowest RWC values [36], [20], [8]. The ability of a plant to tolerate water stress depends on several factors, including its morphology, physiology and biochemistry [20]. Water stress and its effects on plant metabolites using RWC as a guide, give us an insight into the internal water relations of honeybush plants. Therefore, the slight decrease in RWC in plants irrigated twice a week may indicate that *C. subternata* has tolerance for mild to moderate water deficit stress.

Proline

Water stress in plants increases metabolite levels and stimulates metabolism [37]. In the first sampling week, no significant difference ($p > 0.05$) was observed among all the three treatments. Thereafter, the highest proline contents were consistently observed in plant populations that received irrigation once a week as shown in Fig. 1B. Whereas the well-watered *C. subternata* plants had significantly lowest proline accumulation throughout the observation period. Proline accumulation can occur in plants regardless of stress or non-stress conditions but is relatively low under optimal irrigation conditions [38]. In this study, an increase in proline content in *C. subternata* plants indicates higher water stress and vice-versa. In general, as the irrigation treatments progressed, the proline concentration in the control treatment appears to be decreasing while that of the plants under less frequent irrigation appears to be increasing with time. Reference [8] reported similar results in *C. subternata*, where there were massive and slight increases in proline contents in the stressed and semi-stressed treatments respectively. Although some authors question the link between proline accumulation and stress adaptation, it is generally accepted that plant cells benefit from an increase in proline content after injury [16].

Stomatal conductance

In this study, the average stomatal conductance of *C. subternata* as influenced by watering frequency is presented in Fig. 2. No significant difference ($p > 0.05$) was observed in the first three weeks of sampling dates for all irrigation treatments. The reason for this may be because the plants were yet to reach a threshold where stomatal closure is triggered as leaf water potential reaches a critical stress level due to deficit irrigation [39]. However, the stressed plants (watering twice and once a week) generally had lower stomatal conductance, which became significantly lower ($p < 0.05$) in the last two weeks of the study. Although, the two water stressed treatments did not differ significantly from each other during this period. This result is in accordance with the findings of Chowdhury [40], where a greater reduction in photosynthesis and stomatal conductance was observed in the water stressed genotypes of soybean. According to a study by Atteya [41], drier soils resulted in lower stomatal conductance. As noted by Makbul [42], water stress also decreased stomatal conductance in another study of soybean, in which a 42% decrease in stomatal conductance was observed in drought-stressed leaves compared to non-stressed leaves. Another study reported that, soybean leaves adjust their stomatal conductance to maximize water retention during an extended drought, to prevent losing excessive water [43]. Stomatal closure due to water stress in *C. subternata* leaves resulted in lower stomatal conductance. To ensure survival, the *C. subternata* plants under water stress in this study showed a progressive decline in stomatal conductance, as water deficit stress intensified. The decrease in stomatal conductance in the stressed plants, especially towards the end of the study is an indication of vapor pressure deficit. Increases in vapor pressure deficit between leaf and air lead to the partial closure of stomata, thus, decreasing stomatal conductance to prevent excessive dehydration and physiological damage [39].

TABLE I
CHEMICAL AND PHYSICAL PROPERTIES OF THE SOIL MEDIUM USED IN THE STUDY.

Physical properties		Values
Clay (<0.002 mm)		13
Silt (0.002–0.02 mm)		17
Fine sand (0.02–0.2 mm)		33
Medium sand (0.2–0.5 mm)		3
Coarse sand (0.5–2 mm)		35
Stone volume (%)		0.22
Soil textural class		Coarse sandy loam
Chemical properties		
Ex. Cations (cmol (+)/ kg	Na	0.14
	K	0.52
	Ca	4.4
	Mg	1.6
Macronutrients (mg/kg)	NO3	31.3
	P	23.9
	NH4	3.2
	K	203
Base saturation (%)	K	6.97
	Ca	58.99
	Na	1.88
	Mg	21.45
Acid saturation		10.71
pH (KCl)		5.3
Resistance (Ohms)		800

TABLE II:
WEEKLY GROWTH OF C. SUBTERNATA AS INFLUENCED BY WATERING FREQUENCY.

Sampling time (week)	Irrigation	Plant height (cm)	Stem diameter (mm)	Stem circumference (mm)
1`	Control	22.99 a	1.96 b	6.14 b
	Twice a week	23.75 a	2.19 a	6.88 a
	Once a week	22.57 a	2.09 ab	6.57 ab
2	Control	23.09 a	2.0 b	6.26 b
	Twice a week	24.30 a	2.24 a	7.05 a
	Once a week	22.67 a	2.13 ab	6.69 ab
3	Control	26.49 a	2.23 a	7.02 a
	Twice a week	26.63 a	2.21 a	6.95 a
	Once a week	25.31 a	1.99 a	6.24 a
4	Control	27.98 a	2.25 a	7.08 a
	Twice a week	28.35 a	2.25 a	7.07 a
	Stressed	26.28 a	2.00 a	6.28 a
5	Control	31.00 a	2.45 a	7.69 a
	Twice a week	30.10 ab	2.36 a	7.43 a
	Once a week	27.74 b	2.08 b	6.53 b

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ($P \leq 0.05$).

TABLE III:
PERCENTAGE CHANGE IN C. SUBTERNATA GROWTH IN RESPONSE TO DIFFERENT WATERING FREQUENCIES.

Growth parameter	Irrigation	Percentage change (%)
Plant height (cm)	Control	34.87
	Twice a week	26.74
	Once a week	22.91
Stem diameter (mm)	Control	25.20
	Twice a week	7.96
	Once a week	0.57
Circumference (mm)	Control	25.20
	Twice a week	7.97
	Once a week	0.58

TABLE IV:
AVERAGE SHOOT YIELD OF C. SUBTERNATA IN RESPONSE TO DIFFERENT IRRIGATION FREQUENCIES.

Irrigation	Shoot	
	Fresh weight (g)	Dry weight (g)
Control	4.0513	1.3833
Twice a week	4.4960	1.5198
Once a week	4.5798	1.5775

THERE IS NO SIGNIFICANT DIFFERENCE ($P \geq 0.05$) AMONG TREATMENTS DURING SAMPLING TIME.

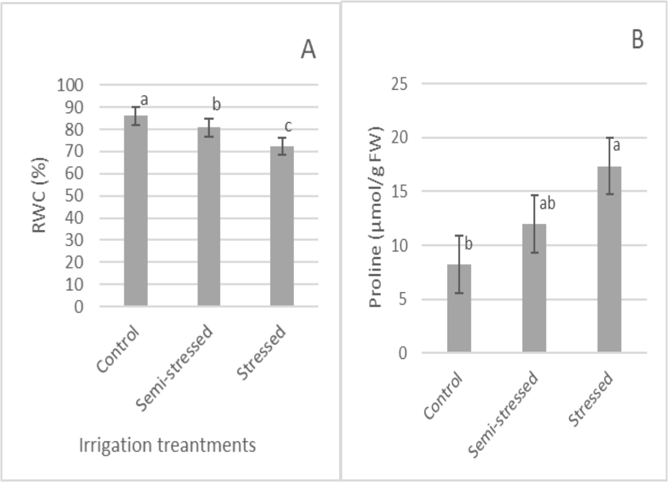


Fig. 1 Effect of diverse water stress levels on relative water content (A) and proline concentration (B) of *C. subternata*.

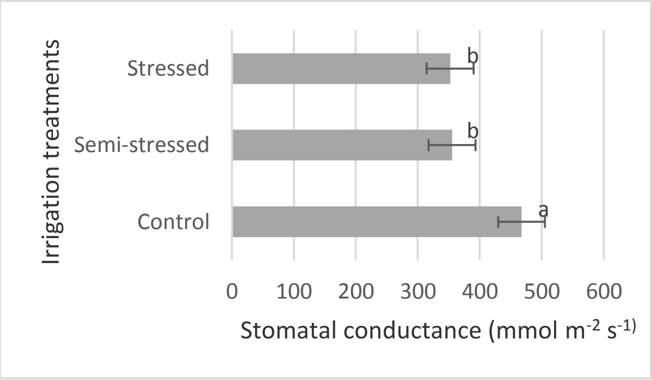


Fig. 2 Effects of irrigation treatments on the stomatal conductance of *C. subternata* leaves.

V.CONCLUSION

Although the irrigation treatments had no significant effect on the overall growth of the *C. subternata* plants, the percentage growth was significantly higher in the well-watered plants compared to the stressed plant populations, indicating better growth and development. Proline concentrations were significantly higher in plants receiving less water than in well-watered plants, which is an indication of water stress. Higher RWC was found in the well-watered plants, followed by plants watered twice a week while the least RWC was observed in the most stressed plants. This proved that proline concentration increases with decreasing RWC and vice versa. Stomatal conductance of plants in this study generally increased with increasing watering frequency and vice versa. The honeybush industry is unable to meet the huge foreign demand that outweighs local supply. Therefore, the cultivation of *Cyclopia* species would not only guarantee the species' sustainability and conservation but will also enhance the standard of living for rural harvesters as well as commercial growers. Irrigation guidelines are critical for this crop as *Cyclopia* species is a niche tea produce.

ACKNOWLEDGMENT

The Department of Science and Innovation (DSI) of South Africa for funding the project. The Department of Higher Learning and Training for supporting my studies financially through Nurturing Emerging Scholar’s Programme (NESP). The staff of Soil Science division, ARC Infruitec-Nietvoorbij for technical support and allowing me to use their facilities. Dr A.T Adetunji for guidance and Dr M. van der Rijst for assistance with statistical analysis.

REFERENCES

[1] Soni, R. P., Katoch, M., Kumar, A., Ladohiya, R., & Verma, P. (2015). Tea: production, composition, consumption and its potential as an antioxidant and antimicrobial agent. International Journal of Food and Fermentation Technology, 5(2), 95-106. https://doi.org/10.5958/2277-9396.2016.00002.7

[2] Van Wyk, B. E., & Gericke, N. (2000). People's plants: A guide to useful plants of Southern Africa. Briza publications.

[3] Joubert, E., Gelderblom, W. C. A., Louw, A., & de Beer, D. (2008). South African herbal teas: Aspalathus linearis, Cyclopia spp. and Athrixia phylicoides—A review. Journal of ethnopharmacology, 119(3), 376-412. https://doi.org/10.1016/j.jep.2008.06.014

[4] Joubert, E., Joubert, M. E., Bester, C., De Beer, D., & De Lange, J. H. (2011). Honeybush (Cyclopia spp.): From local cottage industry to global markets—The catalytic and supporting role of research. South African Journal of Botany, 77(4), 887-907. https://doi.org/10.1016/j.sajb.2011.05.014

[5] Another Joubert

[6] Marnewick, J. L., Gelderblom, W. C., & Joubert, E. (2000). An investigation on the antimutagenic properties of South African herbal teas. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 471(1-2), 157-166. https://doi.org/10.1016/S1383-5718(00)00128-5

[7] Joubert, E., De Beer, D., Malherbe, C. J., Muller, M., Louw, A., & Gelderblom, W. C. A. (2019). Formal honeybush tea industry reaches 20-year milestone–progress of product research targeting phenolic composition, quality and bioactivity. South African Journal of Botany, 127, 58-79 https://doi.org/10.1016/j.sajb.2019.08.027

[8] Mabizela GS, 2020. Metabolic and quality profiling of cyclopia subternata and c. Genistoides in response to seasonal variation and drought stress. Doctoral dissertation, Tshwane University of Technology.

[9] Bester, C. (2012, January). A model for commercialisation of honeybush tea, an indigenous crop. In II All Africa Horticulture Congress 1007 (pp. 889-894). https://doi.org/10.17660/ActaHortic.2013.1007.106

[10] Bester, C., Joubert, M. E., & Joubert, E. (2014, August). A breeding strategy for South African indigenous herbal teas. In XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1127 (pp. 15-22). https://doi.org/10.17660/ActaHortic.2016.1127.3

[11] Department Of Agriculture, Forestry & Fisheries (Daff). 2016. Honeybush tea, production guidelines. [Online]. Available from: https://www.dalrrd.gov.za/Portals/0/Brochures%20and%20Production%20guidelines/Honeybush%20tea%20production%20guideline.pdf [Accessed: 01/05/2022].

[12] Atkinson, N. J., & Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of experimental botany, 63(10), 3523-3543. https://doi.org/10.1093/jxb/ers100

[13] Krasensky, J., & Jonak, C. (2012). Drought, salt, and temperature

stress-induced metabolic rearrangements and regulatory networks. Journal of experimental botany, 63(4), 1593-1608. https://doi.org/10.1093/jxb/err460

[14] Martorell Lliteras, S. (2014). Understanding the regulation of leaf and plant gas exchange under water stress with a process-based model of stomatal conductance.

[15] Brilli, F., Pollastri, S., Raio, A., Baraldi, R., Neri, L., Bartolini, P., ... & Balestrini, R. (2019). Root colonization by Pseudomonas chlororaphis primes tomato (Lycopersicum esculentum) plants for enhanced tolerance to water stress. Journal of plant physiology, 232, 82-93. https://doi.org/10.1016/j.jplph.2018.10.029

[16] Mattioli, R., Costantino, P., & Trovato, M. (2009). Proline accumulation in plants: not only stress. Plant signaling & behavior, 4(11), 1016-1018. https://doi.org/10.4161/psb.4.11.9797

[17] Szepesi, Á., & Szőllősi, R. (2018). Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants. In Plant metabolites and regulation under environmental stress (pp. 337-353). Academic Press. https://doi.org/10.1016/B978-0-12-812689-9.00017-0

[18] Flexas, J., Diaz-Espejo, A., Gago, J., Gallé, A., Galmés, J., Gulías, J., & Medrano, H. (2014). Photosynthetic limitations in Mediterranean plants: a review. Environmental and Experimental Botany, 103, 12-23. https://doi.org/10.1016/j.envexpbot.2013.09.002

[19] Surendar, K. K., Devi, D. D., Ravi, I., Jeyakumar, P., & Velayudham, K. (2013). Water stress affects plant relative water content, soluble protein, total chlorophyll content and yield of ratoon banana. International Journal of Horticulture, 3. https://doi.org/10.5376/ijh.2013.03.0017

[20] Soltys-Kalina, D., Plich, J., Strzelczyk-Żyta, D., Śliwka, J., & Marczewski, W. (2016). The effect of drought stress on the leaf relative water content and tuber yield of a half-sib family of ‘Katahdin’-derived potato cultivars. Breeding science, 66(2), 328-331. https://doi.org/10.1270/jsbbs.66.328

[21] Blum, A. (2011). Plant water relations, plant stress and plant production. In Plant breeding for water-limited environments (pp. 11-52). Springer, New York, NY. https://doi.org/10.1007/978-1-4419-7491-4_2

[22] Lugojan, C., & Ciulca, S. (2011). Evaluation of relative water content in winter wheat. Journal of Horticulture, Forestry and Biotechnology, 15(2), 173-177.

[23] Harb, A., Krishnan, A., Ambavaram, M. M., & Pereira, A. (2010). Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant physiology, 154(3), 1254-1271. https://doi.org/10.1104/pp.110.161752

[24] Bennie, A. T. P., & Hensley, M. (2001). Maximizing precipitation utilization in dryland agriculture in South Africa—a review. Journal of Hydrology, 241(1-2), 124-139. https://doi.org/10.1016/S0022-1694(00)00377-2

[25] Lionello, P., Malanotte-Rizzoli, P., Boscolo, R., Alpert, P., Artale, V., Li, L., ... & Xoplaki, E. (2006). The Mediterranean climate: an overview of the main characteristics and issues. Developments in earth and environmental sciences, 4, 1-26. https://doi.org/10.1016/S1571-9197(06)80003-0

[26] Sade, N., Galkin, E., & Moshelion, M. (2015). Measuring Arabidopsis, tomato and barley leaf relative water content (RWC). Bio-protocol, 5(8), e1451-e1451. https://doi.org/10.21769/BioProtoc.1451

[27] Ábrahám, E., Hourton-Cabassa, C., Erdei, L., & Szabados, L. (2010). Methods for determination of proline in plants. In Plant stress tolerance (pp. 317-331). Humana Press. https://doi.org/10.1007/978-1-60761-702-0_20

[28] Little T. M and Hills F J, 1972. Statistical Methods in Agricultural Experimentation. University of California, Davis. California 95616, pp 93-101.

[29] Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). Biometrika, 52(3/4), 591-611. https://doi.org/10.1093/biomet/52.3-4.591

[30] Ott, R. L., & Longnecker, M. T. (2015). An introduction to statistical methods and data analysis. Cengage Learning.

[31] Tshikhudo, P. P., Ntushelo, K., Kanu, S. A., & Mudau, F. N. (2019). Growth response of bush tea (Athrixia phylocoides DC.) to climatic conditions in Limpopo Province, South Africa. South African Journal of Botany, 121, 500-504. https://doi.org/10.1016/j.sajb.2018.12.012

[32] Hao, X., Wang, L., Zeng, J., Yang, Y., & Wang, X. (2018). Response and adaptation mechanisms of tea plant to low-temperature stress. In Stress physiology of tea in the face of climate change (pp. 39-61). Springer, Singapore. https://doi.org/10.1007/978-981-13-2140-5_3

[33] Habibi, G. (2018). Effects of mild and severe drought stress on the biomass, phenolic compounds production and photochemical activity of Aloe vera (L.) Burm. f. Acta Agriculturae Slovenica, 111(2), 463-476. https://doi.org/10.14720/aas.2018.111.2.19

[34] Eziz, A., Yan, Z., Tian, D., Han, W., Tang, Z., & Fang, J. (2017). Drought effect on plant biomass allocation: A meta-analysis. Ecology and evolution, 7(24), 11002-11010. https://doi.org/10.1002/ece3.3630

[35] Zhao, T. J., Sun, S., Liu, Y., Liu, J. M., Liu, Q., Yan, Y. B., & Zhou, H. M. (2006). Regulating the drought-responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and trans-inactive DRE binding factors in Brassica napus. Journal of Biological Chemistry, 281(16), 10752-10759. https://doi.org/10.1074/jbc.M510535200

[36] Boussadia, O., Mariem, F. B., Mechri, B., Boussetta, W., Braham, M., & El Hadj, S. B. (2008). Response to drought of two olive tree cultivars (cv Koroneki and Meski). Scientia Horticulturae, 116(4), 388-393. https://doi.org/10.1016/j.scienta.2008.02.016

[37] Sharma, H. S., Feng, L., Muresanu, D. F., Castellani, R. J., & Sharma, A. (2019). Neuroprotective effects of a potent bradykinin B2 receptor antagonist HOE-140 on microvascular permeability, blood flow disturbances, edema formation, cell injury and nitric oxide synthase upregulation following trauma to the spinal cord. In International review of neurobiology (Vol. 146, pp. 103-152). Academic Press. https://doi.org/10.1016/bs.irn.2019.06.008

[38] Kavi Kishor, P. B., Hima Kumari, P., Sunita, M. S. L., & Sreenivasulu, N. (2015). Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. Frontiers in Plant Science, 6, 544. https://doi.org/10.3389/fpls.2015.00544

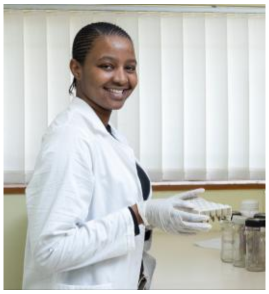
[39] Mofokeng, M. M., Steyn, J. M., Du Plooy, C. P., Prinsloo, G., & Araya, H. T. (2015). Growth of Pelargonium sidoides DC. in response to water and nitrogen level. South African Journal of Botany, 100, 183-189. https://doi.org/10.1016/j.sajb.2015.05.020

[40] Chowdhury, J. A., Karim, M. A., Khaliq, Q. A., Ahmed, A. U., & Khan, M. S. A. (2016). Effect of drought stress on gas exchange characteristics of four soybean genotypes. Bangladesh Journal of Agricultural Research, 41(2), 195-205. https://doi.org/10.3329/bjar.v41i2.28215

[41] Atteya, A. M. (2003). Alteration of water relations and yield of corn genotypes in response to drought stress. Bulg. J. Plant Physiol, 29(1-2), 63-76.

[42] Makbul, S., Saruhan Güler, N., Durmuş, N., & Güven, S. (2011). Changes in anatomical and physiological parameters of soybean under drought stress [Kuraklık stresi altındaki soya fasülyesinin anatomik ve fizyolojik parametrelerindeki değişimler]. https://doi.org/10.3906/bot-1002-7

[43] Ku, Y. S., Au-Yeung, W. K., Yung, Y. L., Li, M. W., Wen, C. Q., Liu, X., & Lam, H. M. (2013). Drought stress and tolerance in soybean. A comprehensive survey of international soybean research—Genetics, physiology, agronomy and nitrogen relationships, 209-237. https://doi.org/10.5772/52945



Mary-Jane Mahlare was born and bred in the rural village of Ga-Marishane in Limpopo, South Africa. She went to Bopedi Bapedi Secondary (2008-2012) then enrolled at Madzivhandila College of Agriculture (2015-2017) where she obtained her Diploma in Agriculture (Plant Production). She was awarded two merit certificates for best plant production student and overall best student (cum laude) by the Limpopo Department of Agriculture and Rural Development. In 2018, she worked as an agricultural advisor intern at Madzivhandila College under the AgriSeta program for 12 months. She then registered for Bachelor of Technology in Agriculture (Crop Production) at Cape Peninsula University of Technology (CPUT) in 2019 and obtained her qualification in the same academic year. During her BTech studies, she worked at Agricultural Research Council (Infruitec - Nietvoorbij) as a research student analysing soil enzyme activity. In January 2020, she made a poster presentation at the Combined Congress, which was held at the University of Free State, Bloemfontein, South Africa. She is currently registered as a master’s student at Cape Peninsula University of Technology (Wellington Campus), doing her research on the water needs of honeybush at ARC (Infruitec - Nietvoorbij). She also presented at Indigenous Plant Use Forum (IPUF) and CPUT conferences in 2021.