PHYSIOLOGY AND ROOT PROFILES OF DOWNY ROSE MYRTLE (*RHODOMYRTUS TOMENTOSA*) GROWN WITH PALM KERNEL BIOCHAR UNDER DIFFERENT WATER HOLDING CAPACITY LEVELS

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Abstract. Water stress significantly limits plant physiology, especially in drought-prone locations. Biochar, such as palm kernel (PK) biochar, enhances soil properties and plant resilience during drought. However, its effects on potential soil-stabilizing plants under water deficit are underexplored. Hence, this study examines the physiological responses and root profiles of *Rhodomyrtus tomentosa* (Downy Rose Myrtle) grown under varying water holding capacities (WHC) (100%, 75%, 50% and 25%) treated with or without 10% (w/w) PK biochar. A six-month shade-house experiment was conducted using a randomized complete block design (RCBD). Biochar-treated plants showed increased photosynthetic rate under water stress, with an increase of 45.27% in plants subjected to 25% WHC. Similarly, improved water use efficiency (WUE) was also observed in biochar-treated plants subjected to 50% and 25% WHC. Root lengths were also significantly increased in biochar-treated plants of 75%, 50% and 25% WHC. Statistical analysis revealed that the interaction between WHC and biochar application significantly affected photosynthetic rate, transpiration rate, WUE and root length, likely attributed to improved soil water availability, nutrient retention contributed by PK biochar, optimizing photosynthesis, gaseous exchange and root development. Overall, PK biochar improved several physiological responses and root profiles of *R. tomentosa* and may serve as a viable soil amendment for vegetation, such as *R. tomentosa* in water deficit conditions.

Keywords: *shrubs, soil amendment, water stress, plant physiology, drought*

Introduction

The global climate crisis has been accelerating at an unprecedented rate over the past few decades. Extreme climatic events, including heat waves and droughts have become more prevalent and are expected to become more frequent and intense in the future (Lloret et al., 2012). An increase in global temperatures and evident decrease in rainfall has left an adverse effect on growth and productivity of plants due to an increase in evapotranspiration, leading to temperature and water stress (Chen et al., 2023). In plants, water stress is categorized as one of the devastating abiotic stressors that leads to a decrease in plant growth and productivity, more than half of its usual capability (Mishra et al., 2023). Depreciation of cell membrane integrity and a reduction in turgor pressure of cells will eventually lead to stomatal closure, which in turn reduces gaseous exchange in plant cells and subsequently reduces photosynthetic efficiency (Kim et al., 2021).

Plants play an important role in maintaining the balance in the environmental ecosystem such as stability of soil structures especially in hills and mountains. However, rapid urbanization, coupled with worrying levels of climate change, poses a threat to

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developing countries, where finding a compromise between development and minimizing detrimental impacts is paramount. Vegetation offers a natural alternative to achieving structural stability, through eco-engineering. Eco-engineering refers to ecological solution, in this case, controlling soil erosion that are more suited for long-term results (Mickovski and Van Beek, 2007). With vegetations, self-repairing trait and reinforcement effect of roots help binding soil together, directly increasing soil stability (Tardío and Mickovski, 2016). Nonetheless, without appropriate vegetation that could withstand drought conditions and able to oppose effects of water stress that may be faced, this objective could not be achieved.

Soil amendments, such as biochar, has garnered traction in the past decade, to improve soil and living conditions for organisms in soil. Biochar is a porous carbonaceous material that are produced through pyrolysis process (at 250-700°C) of organic materials such as agricultural wastes and bear similarities to charcoal (Wu et al., 2023). Numerous studies have shown that biochar is beneficial to plants, in terms of plant growth, productivity, biomass, nutrient uptake and gaseous exchange (Mansoor et al., 2021). This can be achieved through porous biochar properties that subsequently increases water holding capacity of soil, which allows available water for plants to be retained in the soil (Mansoor et al., 2021). In turn, it reduces effects of stressors such as water stress that is known to reduce efficacy of photosynthesis by the enzyme RuBisCO in photorespiration, in addition to improving plants’ xylem water potential, chlorophyll content and stomatal conductance in leaves (Mansoor et al., 2021). Furthermore, biochar can promote soil fertility and provide a habitat for soil microbes to colonize, reproduce and grow which contributes to better soil health, subsequently contributing to better plant performance (Kong et al., 2019; Wu et al., 2023). Thus, biochar could be an important “cofactor” to maintain, as well as, improving plant performance especially during water deficit conditions.

Rhodomyrtus tomentosa are amongst several potential candidates of slope plants, which aims to improve slope stability. Downy rose-myrtle or known locally as Kemunting is a type of flowering shrub species under *Myrtaceae* family (Winotai et al., 2009). *R. tomentosa* is one of the fast-growing evergreen shrubs that is native in the south and south-eastern Asia, such as in Malaysia, Indonesia, Thailand and Vietnam (Csurhes and Hankamer, 2016; Vo and Ngo, 2019). In previous studies, *R. tomentosa* possesses several pharmaceutical properties, including anti-inflammatory antioxidant properties (Vo and Ngo, 2019). However, research on the physical and physiological properties that define the species are severely underexplored. There have been observations that *R. tomentosa* was able to survive in the wild, rough conditions such as coastal and degraded shrubland slopes but have not been researched further (Wagner et al., 1999; Wei et al., 2009; Yang et al., 2010). Physically, *R. tomentosa* can be characterized by possessing small-sized and waxy leaves, which could potentially contribute to good water use efficiency (WUE) and improving its ability to survive in dry and arid environments. Furthermore, this attribute would be able to support *R. tomentosa* as a potential plant that can be used to improve soil stability through its rooting systems, in the case of dry conditions. Hence, the current study is aimed to investigate the effect of biochar on plant growth, soil properties and root profiles of *R. tomentosa* grown under water deficit conditions.

Materials and methods

Site description

This study was conducted in a shade house located in Rimba Ilmu, Institute of Biological Sciences, Universiti Malaya, Kuala Lumpur, Malaysia from August 2023 to January 2024. The ambient temperature in the shade house varied from 25 to 32 °C, whilst the relative humidity (RH), minimum and maximum photosynthetically active radiation (PAR) recorded was 60-90%, 100 μE mol m^{-2} s^{-1} and 2100 μE mol m^{-2} s^{-1}, respectively.

Experimental design and plant materials

Four (4)-month old *R. tomentosa* seedlings of similar height (± 5 cm) were obtained from a nursery in Sungai Buloh, Selangor, Malaysia. A total of 64 seedlings were transplanted into individual polybags of 12 x 15 inch (30.47 x 38.1 cm). Polybag holes were sealed with clear tape and plastic wrap to minimize water seepage and maintain the applied WHC, particularly at 100% and 75%. Topsoil samples from a slope area in Gamuda Garden, Rawang were used, with soil pH of 4.5 to 5.5. Soils were dried and sieved using a 4.0 mm laboratory sieve to remove larger gravel, rocks and dead plant materials.

Palm kernel (PK) biochar, obtained from Malaysian Palm Oil Board (MPOB), was used as a soil amendment in this study. PK biochar was ground and sieved through 2.0 mm sieve and mixed thoroughly with sieved soil 15 days prior to transplanting. Physicochemical properties of PK biochar are outlined in *Table 1*, the same PK biochar used by research on acidic soil by Rusli et al. (2021). Flat percentage of 10% biochar (w/w) to soil weight was applied, which was reported to be optimal for good and optimal water retention in soil by Ibrahim et al. (2013), which is detrimental to prevent compromise to plant and soil structure. An average 8 kg of soil (control) and soil mixture (soil with biochar) were measured per polybag that is used to transplant *R. tomentosa* for this study. Additionally, 5 g of NPK Green (15:15:15) fertilizer were applied every four (4) weeks to both control and biochar treatments to replenish nutrients from being planting in polybags, adapting this routine from research on *S. campalunatum* by Mohd Roseli et al. (2010).

Table 1. *Physico-chemical properties of soil and palm kernel biochar used*

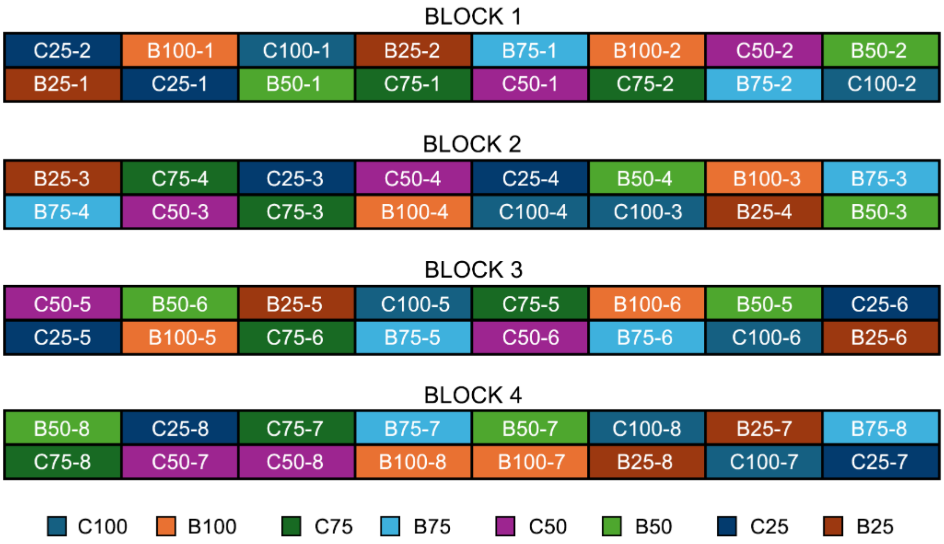
Properties	Soil	PK Biochar
pH	5.12	8.61
Total OC (%)	0.08	43.31
N (%)	0.03	0.50
Available P (mg/kg)	88	0.15
K (meq/100 g)	0.346	0.74
Ca (meq/100 g)	0.61	2.27
Mg (meq/100 g)	0.074	0.25

Seedlings were grown under the combination of two main variables, water holding capacities (WHC; 25%, 50%, 75% and 100%) and biochar application, outlined in *Table 2*. A 5% WHC margin range from the original WHC is set, similar to Shang et al. (2024), to account for variability, such as evapotranspiration and percolation of water down the potted soil. Treatments were ensured to possess moisture levels below its respective WHC range before rewatering. Plants were arranged in a Randomized

Complete Block Design (RCBD), totalling four (4) blocks. Each block contained all eight (8) treatments with two (2) replicates each, for a total of 64 plants in all four (4) blocks, outlined in *Figure 1*. Soil WHC% of each treatment was measured and maintained throughout experimental period of six (6) months, by measurement and watering every two- to three-day interval and/or after soil moisture drops below its appropriate soil water holding capacity, which are 100%, 75%, 50% and 25% of WHC.

Table 2. *Treatments of experimental planting. Letters indicate types of treatment; C=control, B=biochar*

No.	Biochar Application	Water Holding Capacity (%)	Treatment Label	Stress Category
1	No (Control)	95-100	C100	Waterlogged
2	Yes (10%)		B100	
3	No (Control)	70-75	C75	Control
4	Yes (10%)		B75	
5	No (Control)	45-50	C50	Mild
6	Yes (10%)		B50	
7	No (Control)	20-25	C25	Severe
8	Yes (10%)		B25	



BLOCK 1

C25-2	B100-1	C100-1	B25-2	B75-1	B100-2	C50-2	B50-2
B25-1	C25-1	B50-1	C75-1	C50-1	C75-2	B75-2	C100-2

BLOCK 2

B25-3	C75-4	C25-3	C50-4	C25-4	B50-4	B100-3	B75-3
B75-4	C50-3	C75-3	B100-4	C100-4	C100-3	B25-4	B50-3

BLOCK 3

C50-5	B50-6	B25-5	C100-5	C75-5	B100-6	B50-5	C25-6
C25-5	B100-5	C75-6	B75-5	C50-6	B75-6	C100-6	B25-6

BLOCK 4

B50-8	C25-8	C75-7	B75-7	B50-7	C100-8	B25-7	B75-8
C75-8	C50-7	C50-8	B100-8	B100-7	B25-8	C100-7	C25-7

■ C100 ■ B100 ■ C75 ■ B75 ■ C50 ■ B50 ■ C25 ■ B25

Figure 1. *Arrangement of Randomized Complete Block Design (RCBD) of experimental planting involving 64 plants, 8 replicates per treatment. Letters indicate types of treatment; C=control, B=biochar*

Soil moisture was determined using a portable HH2 Moisture Meter (Delta-T Devices Ltd., Cambridge, UK) by inserting sensor probe at 3-6 cm soil depth. To determine WHC, soil and biochar-treated soil was saturated with water for 48 h. After 48 h, 300 g of saturated soil and soil mixture, A from middle section of the pot were harvested, then oven-dried at 90°C for 72 h to obtain dry weight, B. WHC was determined through gravimetric method outlined by FAO (2023) through the formula:

$$\mathrm{WHC}\ (\%) = [(A - B)\ \mathrm{x}\ 100]/B \qquad (\mathrm{Eq.1})$$

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75, 50 and 25% WHC were then calculated subsequently, presented in *Table 3*. Moisture content of saturated soil was also measured using a moisture meter, representing the moisture content required for soil to reach 100% WHC that is needed to be maintained throughout the experimental planting period. Measured soil moisture content which represents WHC% of control and biochar-treated soil used in this experiment are highlighted in *Table 4*.

Table 3. *Water holding capacity of soil used in the experiment*

Relative Water Holding Capacity	Actual Soil WHC %	
	Control	Biochar-treated
100%	41.51	47.78
75%	31.13	35.83
50%	20.76	23.89
25%	10.38	11.95

Table 4. *Expected soil moisture content reading through HH2 Moisture Meter of corresponding WHC treatments*

Water Holding Capacity	Expected Soil Moisture Content (%vol)	
	Control	Biochar-treated
100%	39.2	42.5
75%	29.4	31.9
50%	19.6	21.3
25%	9.8	10.6

Measured parameters

Plants were monitored immediately after transplanting, to maintain WHC according to respective treatments, and most data involving the plants were collected accordingly throughout the six months of experimental planting.

Relative chlorophyll content

Relative chlorophyll content was measured using a chlorophyll meter (SPAD-502 PLUS, Minolta, Japan). Readings were taken twice a month, on week two and week four, between 1000 - 1230 h, in which photosynthesis takes place the most within the plant. Two replications of readings of relative chlorophyll were measured and average was determined.

Photosynthetic rate, stomatal conductance and transpiration rate

Portable photosynthesis measurement system (LI-6400/XT, LI-COR, USA) was used to measure photosynthetic rate, stomatal conductance and transpiration rate of the plants through its leaves. Measurement was conducted on matured and fully expanded leaves between 1000 h and 1230 h, where photosynthesis theoretically occurs at its peak. Three technical replications were logged simultaneously into the system and recorded for all treatments and their replications. Measurements took place twice a month, in week two and four, for the six-month duration period. Settings of the LI-6400/XT system during measurement are as follows: CO_2 mixer; 400 μmol mol^{-1}, PAR; 1500 μE m^{-2} s^{-1}, Flow rate; 500 μmol/s, Leaf block temperature; 25°C.

Plant water use efficiency

Plant Water Use Efficiency (WUE) is generally termed as instantaneous WUE, can be calculated as net photosynthetic rate divided by transpiration rate (Hatfield and Dold, 2019). There is also another proposed definition, termed as intrinsic WUE, as assimilation of CO_2 and/or dry matter produced per unit mass of water that is used by the plant (Sun et al., 2024). In this research, the value is derived by using the following formula of instantaneous WUE by Hatfield and Dold (2019):

$$WUE = \frac{Photosynthetic\ Rate\ (A)}{Transpiration\ Rate\ (E)} \qquad \text{(Eq.2)}$$

Leaf relative water content

Two of the youngest, fully expanded leaves from the middle section of the branch were collected and weighed immediately to determine fresh weight (FW). Each leaf was then floated in a petri dish filled with distilled water and left for 12 h under a fluorescent lamp which supplies light to compensate for sunlight. Leaves were gently blot dry with tissue paper and weighed to obtain turgid weight (TW) before oven dried at 80 °C for 48 h or until weight is constant to obtain dry weight (DW). Relative water content is calculated using the formula outlined by Fariñas et al. (2019) with slight modification, through conversion to percentage:

$$RWC\ (\%) = [\frac{FW - DW}{TW - DW}] \times 100\% \qquad \text{(Eq.3)}$$

Leaf absolute water content

Two of the youngest, fully expanded leaves from the middle section of the branch were collected and weighed immediately to determine fresh weight (FW). The leaves were then dried in oven at 80 °C for 48 h or until weight is constant, to obtain dry weight (DW). Absolute water content is calculated using the formula, also known as leaf water content, outlined by Johnson (2023):

$$AWC\ (\%) = \frac{FW - DW}{FW} \times 100\% \qquad \text{(Eq.4)}$$

Leaf water potential

Leaf water potential was measured using a Scholander Bomb. Branches of *R. tomentosa* were cut at an angle and placed in a sealed zip lock bag to avoid moisture loss to the environment. The branch of the plant was placed in the Scholander Bomb pressure chamber with the end part of the stem facing outwards and outside the pressure chamber. Scholander Bomb pressure chamber is sealed shut before slowly increasing pressure in the chamber with nitrogen gas until water sap is visibly observed on the cut part of the stem. The amount of pressure required to force water sap out of the xylem of the branch was recorded from the pressure meter on the Scholander bomb.

Plant height

Plant heights were measured fortnightly, using a meter ruler, from the base of the stem to the highest point of the plant.

Plant biomass

Plants were harvested after six months of experimental planting period. The leaves, stems and roots of *R. tomentosa* were separated and then oven-dried at 65 °C for 48 h or until constant weight was obtained. Biomass represents dry weight (DW) of the parts harvested.

Root profile

Multiple root measure was carried out using an automated root scanner and imaging system (WinRHIZO Pro v. 2008a, Regent Instruments Inc., Canada). Harvested roots were gently removed from soil and washed under tap water on a 2.0 mm laboratory sieve. Roots were then stored in a 4 °C refrigerator to ensure minimal water droplets observed during root scanning that may interfere during scanning program. Through the automated root scanner, the root length, diameter and volume of the roots can be determined. Diameter of the root was determined by dividing the analysed area of scanned root by its total length while root length was calculated by multiplying the pixel number in a one-pixel thinned image by the pixel size, root volume was determined by calculating the root surface area and length, while root surface area was determined by calculating the root diameter and length (Piñuela et al., 2020). Root length density is calculated through the formula:

$$Root\ Length\ Density\ (RLD) = \frac{Root\ Length}{Soil\ Volume}\% \qquad \text{(Eq.5)}$$

Statistical analysis

Results were analysed through IBM SPSS 26.0 (SPSS Institute, USA). Statistical differences between different treatments were assessed through One-way Analysis of Variance (ANOVA). Duncan’s Multiple Range Test (DMRT) was set for post-hoc mean comparison at 0.05 significance to assess the significant differences in measured treatments, denoted with letters. Student’s t-test was also conducted at ($p \leq 0.05$) to assess statistical significance between treatments within the same water holding capacity, which are denoted with an asterisk symbol (*). Correlation between parameters were analyzed using Pearson’s Correlation Coefficient through XLSTAT 2019.2.2 (Addinsoft, France). Multivariate ANOVA was also conducted, using Hotelling-Lawley’s test to assess the overall interaction effect of WHC and biochar application towards parameters measured in this study. Two-way ANOVA was then conducted to evaluate the interaction effect of WHC and biochar application towards each physiological response, morphology and biomass of *R. tomentosa*.

Results

Relative chlorophyll content

Relative chlorophyll content of *R. tomentosa* in all treatments (*Fig. 2*) generally decreased within the first two weeks of water stress and majority of treatments significantly dropped in the fourth week of treatment. Gradually, relative chlorophyll content recovered until 10th week of treatment and levelled at the end of the observation period. Overall, the relative chlorophyll content of all treatments is considered high, nearing 50 SPAD unit and above, resulting in rich green leaf colour. When comparing between soil treatments, B25 yielded the highest average amongst all treatments, recording 50.5 SPAD unit, increased by 1.66% compared to the control plants at the same WHC level; C25. Additionally, the highest increase was achieved by B75, by 7.18% of control, C75. However, there were no statistically significant differences between biochar and control, in each WHC level.

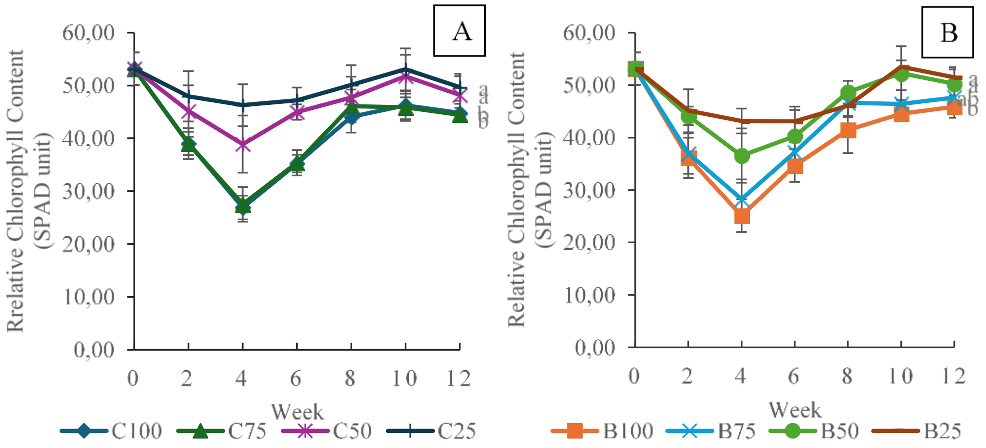

***Figure 2.** Relative chlorophyll content of R. tomentosa. (n=8). (a) Control treatments, (b) Biochar treatments. Vertical bars represent standard deviation. Different letters indicate significant differences at (p ≤ 0.05) between treatments in week 12. C = control, B = biochar, Numbers = WHC*

Photosynthetic rate, stomatal conductance and transpiration rate

General physiological responses such as photosynthetic rates (*Fig. 3*), stomatal conductance (*Fig. 4*) and transpiration rate (*Fig. 5*) were adversely affected in water-stressed *R. tomentosa*. Majority of the biochar treatments yielded a positive increase in photosynthetic rate compared to control, such as in B75 plants, which showed an increased photosynthetic rate (by 33.93%) compared to control plants of the same WHC level; C75. At critical WHC, B25 plants also showed increase photosynthetic rate (by 45.27%) compared to its control plants of the same WHC level; C25. Furthermore, plants under biochar treatment in lower WHCs showed a small increase (by 0.28%) in photosynthetic rate, from B50 to B25. However, plants within control treatments of the same WHC levels showed significantly decreased photosynthetic rate (by 27.85%), from C50 to C25. There were no significant differences observed for transpiration rates in plants grown under 100% WHC, in both control and biochar-treated soil. Overall, photosynthetic rates of plants grown in biochar treatment were significantly higher than plants grown in soil without biochar, except for 50% WHC.

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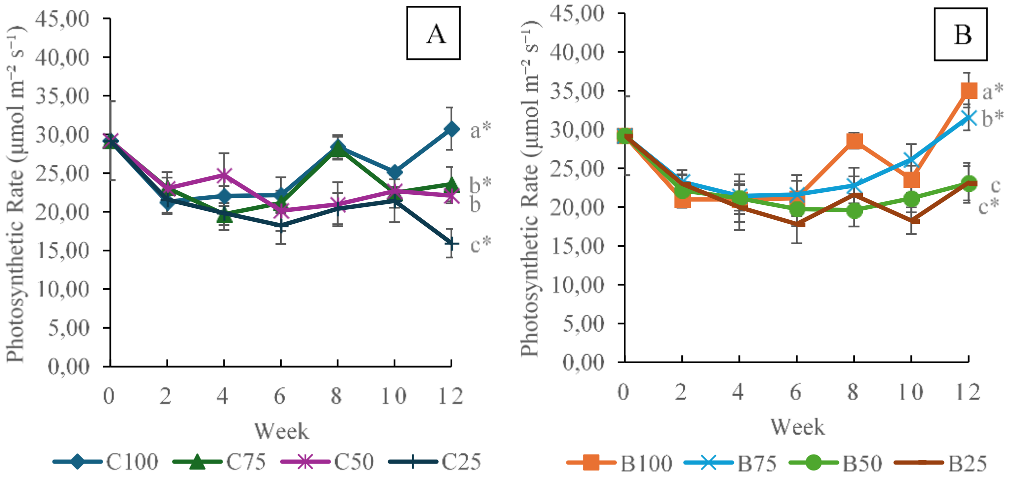

***Figure 3.** Photosynthetic rate of R. tomentosa over 12 weeks. (n=8). (a) Control treatments, (b) Biochar treatments. Vertical bars represent standard deviation. Different letters indicate significant differences at (p ≤ 0.05) between treatments in week 12. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at (p ≤ 0.05); C = control, B = biochar, Numbers = WHC*

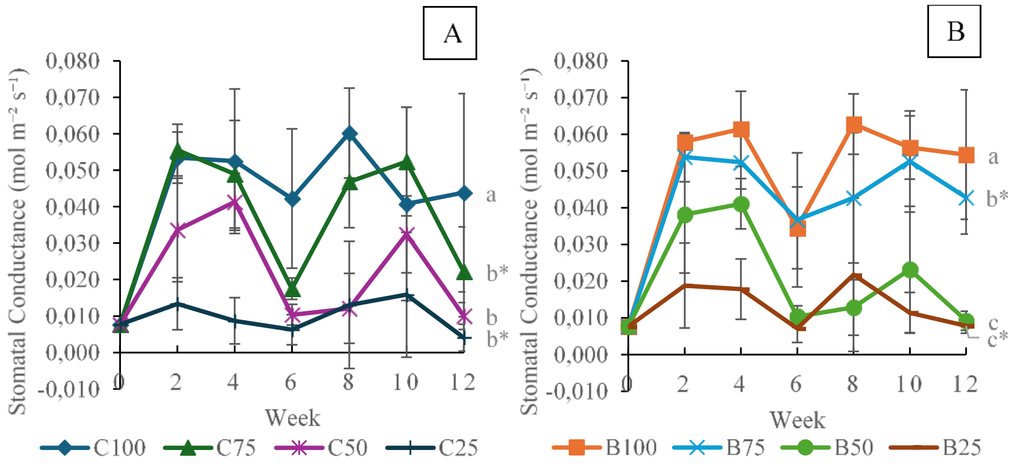

***Figure 4.** Stomatal conductance of R. tomentosa over 12 weeks. (n=8). (a) Control treatments (b) Biochar treatments. Vertical bars represent standard deviation. Different letters indicate significant differences at (p ≤ 0.05) between treatments in week 12. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at (p ≤ 0.05); C = control, B = biochar, Numbers = WHC*

At lower WHCs, biochar-treated plants were observed to result in a significant increase in stomatal conductance (by 92.40%), as shown by B25 plants compared to the control treatment, C25. Biochar-treated plants were also observed to have smaller drop in stomatal conductance (by 15.07%) from B50 to B25, compared to control treatments of similar WHC, from C50 to C25 (by 59.40%). At higher WHCs, biochar-treated plants under 75% WHC; B75 also yielded higher stomatal conductance (by 93.12%) over its control of same WHC; C75. Biochar-treated plants under 100% WHC; B100 also achieved higher stomatal conductance (by 24.20%) over control treatment; C100 but was

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not statistically significant. Overall, plants under biochar treatments exhibited significantly higher stomatal conductance compared to control, in majority of WHC treatment tested, except for 100% and 50% WHC.

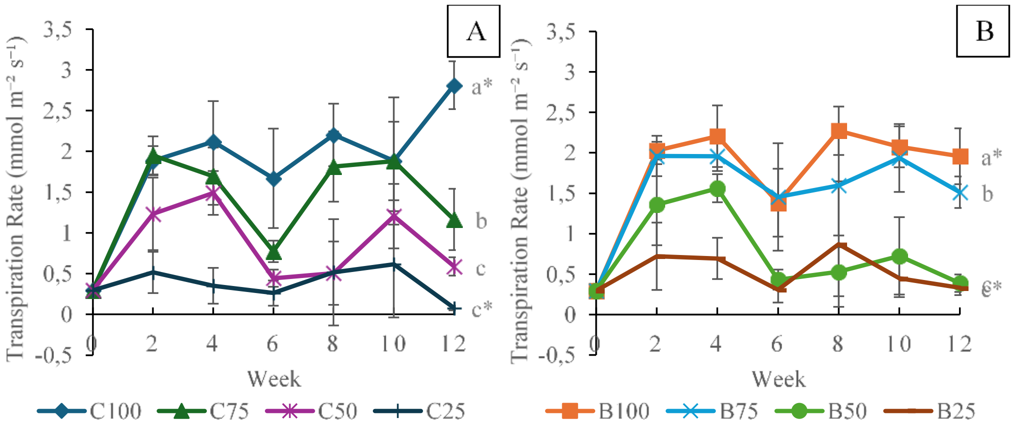

Figure 5. *Transpiration rate of R. tomentosa over 12 weeks. (n=8). (a) Control treatments (b) Biochar treatments. Vertical bars represent standard deviation. Different letters indicate significant differences at ($p \leq 0.05$) between treatments in week 12. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at ($p \leq 0.05$); C = control, B = biochar, Numbers = WHC*

Biochar-treated plants, particularly at 25% WHC; B25 yielded significantly higher transpiration rates (by 315.40%), compared to its control of the same WHC, C25. However, it was also observed that transpiration rate of biochar-treated plants under 50% WHC; B50 were instead significantly lower (by 33.94%) compared to its control of same WHC; C50. Notably, at higher WHC, particularly biochar-treated plants at 75% WHC; B75 had shown an increased transpiration rate (by 29.96%) over control of the same WHC; C75 but was statistically insignificant. On the contrary, control plants under 100% WHC; C100 achieved the overall highest transpiration rate, and significantly higher (by 43.54%) than biochar-treated plants of the same WHC; B100. Overall, the transpiration rates exhibited by plants treated with biochar were not significantly higher than control, except for B25.

Water use efficiency (WUE)

Water use efficiency average across 12 weeks of plants is displayed in *Figure 6*. At higher WHC (100% and 75% WHC), both the control and biochar-treated plants recorded similar WUE, with no significant differences Additionally, these treatments were measured to be the lowest across all treatments compared to lower WHC treatments. Biochar-treated plants that were grown at 25% WHC; B25 achieved the highest WUE overall and were observed to exhibit significantly increased WUE (by 65.23%), compared to control of the same WHC; C25. Furthermore, biochar-treated plants under 50% WHC; B50 also showed significantly higher WUE (by 22.42%) compared to control of same WHC; C25. Overall, the WUE recorded in biochar treatments was comparable and not significantly different than that showed by control plants (subjected to the same WHC level), except for 25 WHC%.

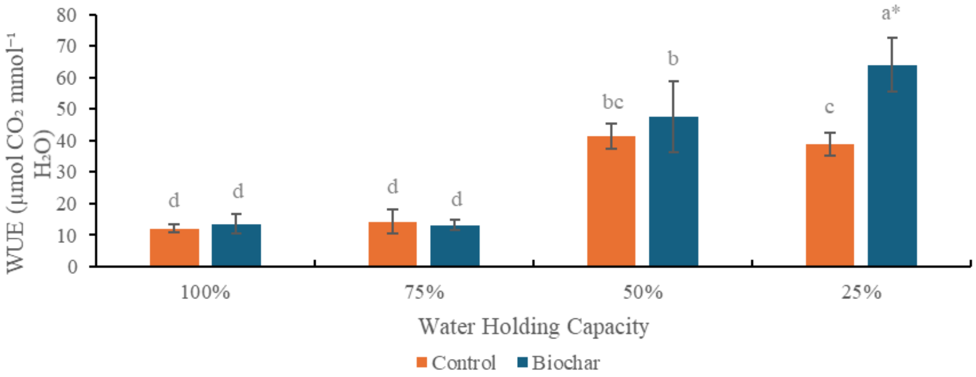

Figure 6. *Average water use efficiency of R. tomentosa average over 12 weeks. (n=8). Vertical bars represent standard deviation. Different letters indicate significant differences at (p ≤ 0.05) between treatments across 12 weeks. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at (p ≤ 0.05)*

Leaf relative water content

Measurements of leaf relative water content in three (3) separate weeks across 12 weeks of treatment (*Fig. 7*) had indicated that leaf relative water content had remained relatively consistent throughout the treatment period, until week 12. On week 12, average leaf water content of plants grown under higher WHC; 100% and 75% WHC, for both control and biochar-treated plants, was significantly higher than plants subjected to lower WHCs. Biochar-treated plants under 50% WHC; B50, showed an increase in RWC (by 6.73%) compared to control of the same WHC, C50. Notably, RWC of biochar-treated plants in 25% WHC; B25 were comparable to biochar-treated plants of 50% WHC; B50, and relatively higher (by 3.72%) than control treatment of the same WHC; C50.

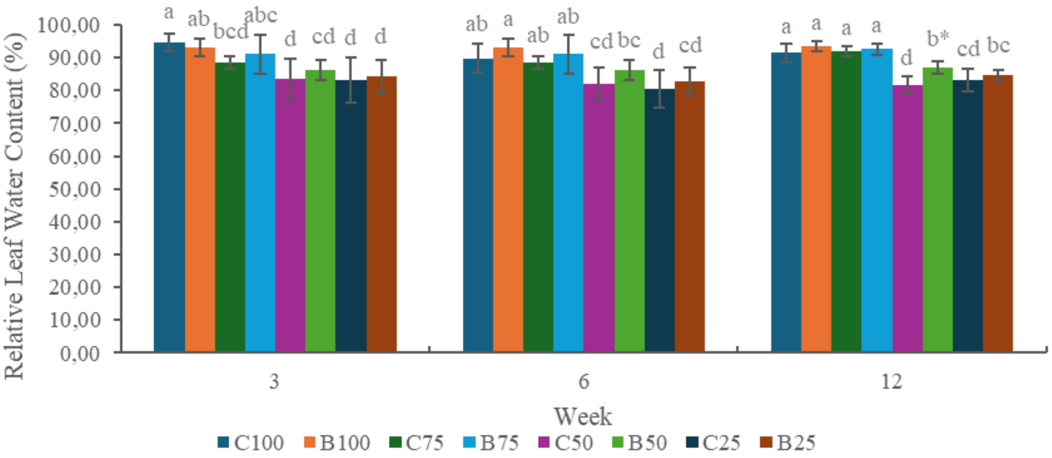

Figure 7. *Leaf relative water content of R. tomentosa over 12 weeks. (n=8). Vertical bars represent standard deviation. Different letters indicate significant differences at (p ≤ 0.05) between treatments; Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at (p ≤ 0.05), C = control, B = biochar, Numbers = WHC*

Leaf absolute water content

For leaf absolute water content (AWC) of *R. tomentosa* (*Fig. 8*), there were significant differences between higher and lower WHC treatments. For plants grown under higher WHC, there were no significant differences in AWC, except for biochar-treated plants grown under 75% WHC; B75 which yielded significantly higher AWC (by 5.30%) than control plants of the same WHC; C75. At lower WHC, biochar-treated plants under 25% WHC, B25; had significantly higher AWC (by 13.04%) than control plants of the same WHC; C25. Notably, AWC of biochar-treated plants grown under 50% WHC; B50 were comparable to control plants of the same and lower WHC; C50 and C25. Overall, biochar treatment only shown significant increases in AWC over control, under 75% and 25% WHC.

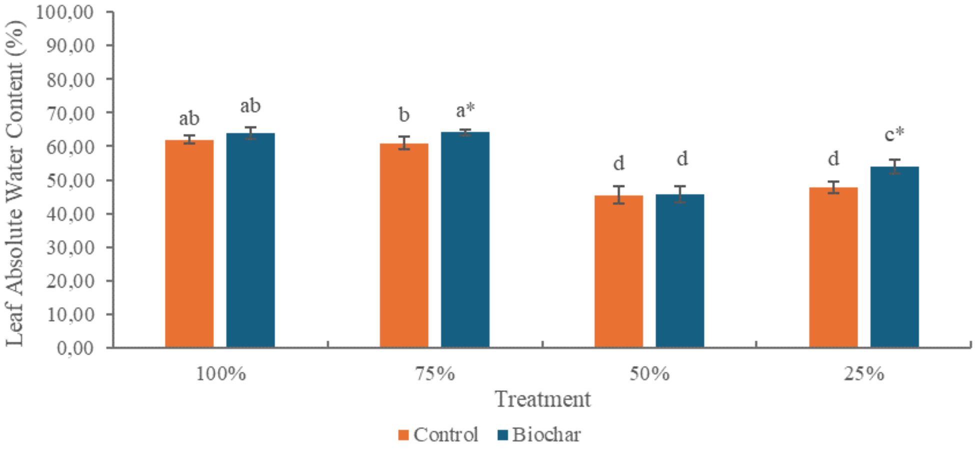

Figure 8. *Leaf absolute water content of R. tomentosa during week 12. (n=8). Vertical bars represent standard deviation. Different letters indicate significant differences at (p ≤ 0.05) between treatments. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at (p ≤ 0.05)*

Leaf water potential

Leaf water potential (LWP) results of *R. tomentosa* (*Fig. 9*) showed that the water potential of plants measured decreased accordingly, from high to low WHC for both control and biochar-treated plants. At high WHC, there were no significant differences observed within treatments, even with biochar treatment. However, LWP of biochar-treated plants grown under both 100% and 75% WHC; B100 and B75 was observed to be lower (by 13.32% and 8.48%, respectively), compared to control plants of the same WHC; C100 and C75, but the differences were statistically insignificant. At lower WHCs, there were also no significant differences observed between LWP of control and biochar-treated plants, and between plants grown in different WHCs.

Plant height

Plant height increment of *R. tomentosa* (*Fig. 10*) measured throughout the experimental planting had indicated that the increase in plant height was significantly affected when the plants were faced with mild and severe water stress conditions. Most biochar-treated plants (*Fig. 10b*), including plants grown under lower WHCs, such as B25, exhibited higher plant height increment (by 38.90%) compared to control plants of the same WHC; C25. At higher WHCs, biochar contribution could also be observed, as biochar-treated plants grown under 75% WHC; B75 achieved the highest average plant height increment, over all treatments, including C100 and B100, and compared to control plants of the same WHC; C75 (by 16.15%). However, overall, the height increment biochar-treated plants were not statistically significant over control plants of each WHCs tested, even though higher height increment was observed in several WHCs, such as B75 and B25.

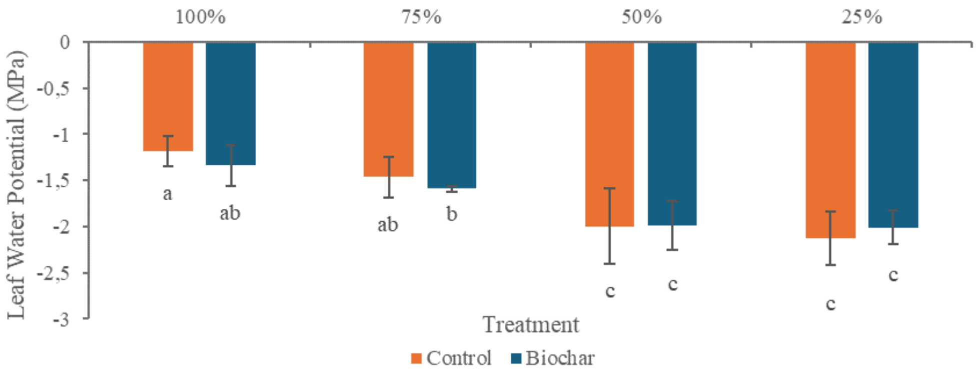

Figure 9. *Leaf water potential of R. tomentosa. (n=8). Vertical bars represent standard deviation. Different letters indicate significant differences at ($p \leq 0.05$) between treatments. Negative values indicate pressure applied is against force of gravity*

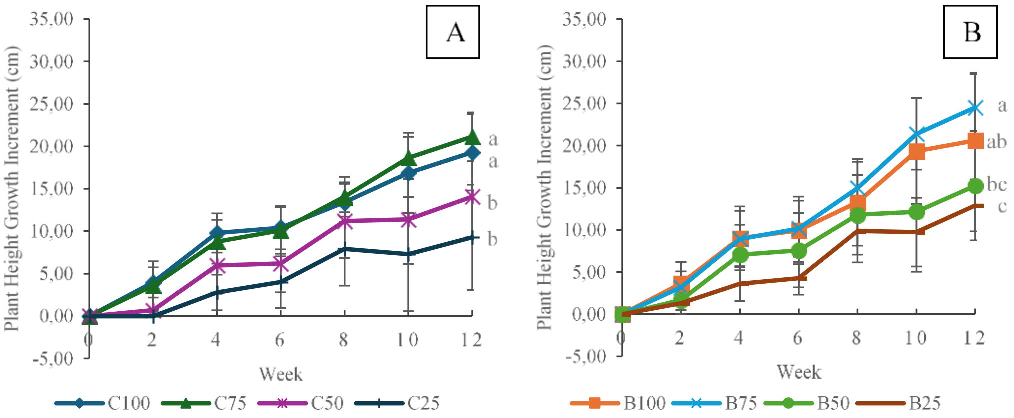

Figure 10. *Plant height increment of R. tomentosa. (n=8). (a) Control treatments, (b) Biochar treatments. Vertical bars represent standard deviation. Different letters indicate significant differences at ($p \leq 0.05$) between treatments in week 12.; C = control, B = biochar, Numbers = WHC*

Plant biomass

According to *Fig. 11a*, biochar-treated plants exhibited a slight increase in branch dry biomass over control plants of the same WHC, except for biochar-treated plants grown under 75% WHC; B75. B75 measured the highest average branch dry biomass across all treatments, only second to control plants grown under 50% WHC; C50. At lower WHC, biochar-treated plants grown under 50% WHC; B50 branch dry biomass was significantly lower (by 27.19%) than control plants of the same WHC, C50. Additionally, branch dry biomass of biochar-treated plants under 25 WHC%; B25 did not have significant difference to control plants of the same WHC; C25.

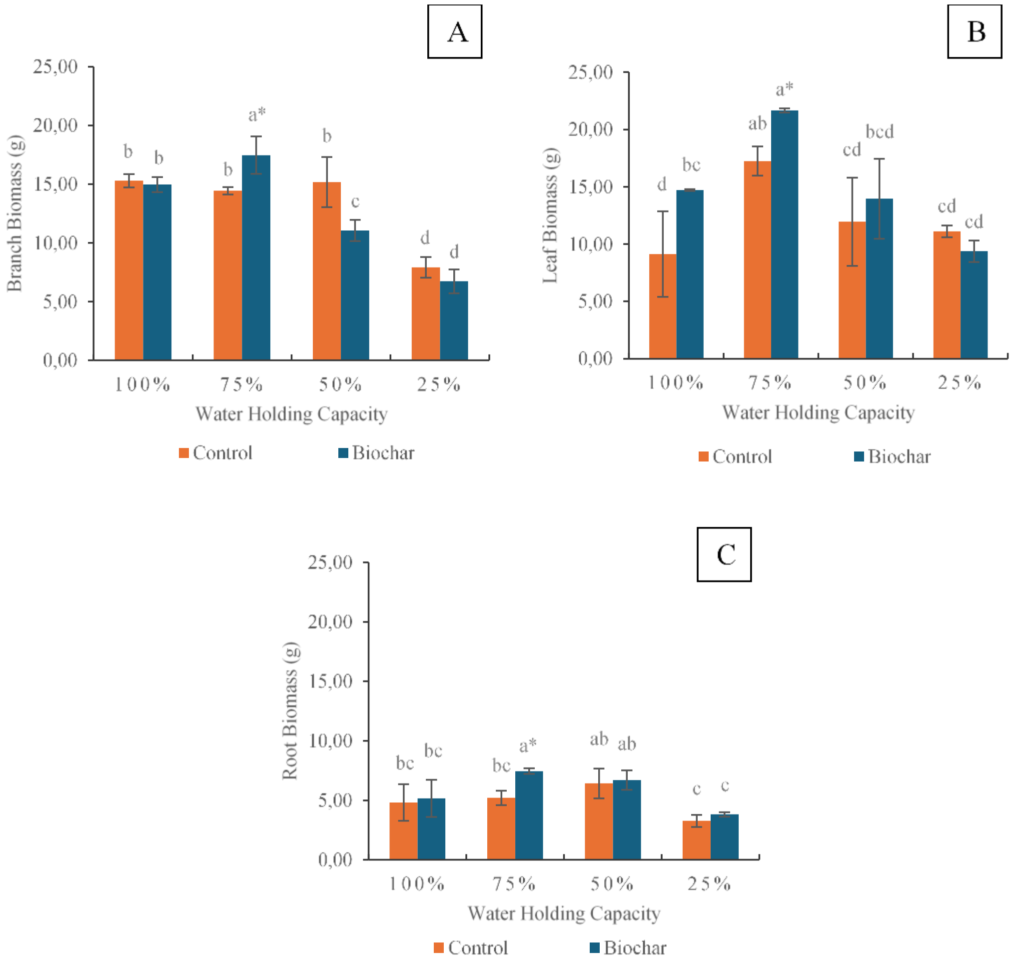

__Figure 11.__ Average dry plant biomass parts of R. tomentosa. (n=8). (a) Branch biomass. (b) Leaf biomass. (c) Root biomass. Vertical bars represent standard deviation. Different letters indicate significant differences at ($p \leq 0.05$) between treatments. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at ($p \leq 0.05$)

Similar results were observed in leaf and root dry biomass. In terms of leaf dry biomass (*Fig. 11b*), biochar-treated plants grown under 75% WHC; B75 had the highest average leaf dry biomass over other treatments. However, leaf dry biomass of biochar-treated plants of 100% WHC; B100 was significantly higher (by 60.89%) than the control plants subjected to the same WHC, C100. Furthermore, biochar-treated plants of 50% WHC; B50 had higher average leaf biomass (by 16.86%) compared to control plants of the same WHC; C50 but it is not statistically significant. Notably, the average leaf dry biomass of biochar-treated plants grown under 50% WHC; B50 were also comparable to both control and biochar-treated plants of 100%, 75% and 25% WHC.

For root dry biomass (*Fig. 11c*), biochar-treated plants grown under 75% WHC; B75 were also observed to be the highest out of all treatments and were significantly higher (by 43.10%) than control plants of the same WHC; C75. Additionally, dry root biomass of biochar-treated plants of 75% WHC; B75 was also significantly higher (by 44.10%

and 54.35%) than both plants grown on 100% WHC, for B100 and C100, respectively. Both root dry biomass of plants grown under 50% WHC; C50 and B50 were also comparable to biochar-treated plant grown under 75% WHC; B75. Although root dry biomass was significantly reduced in lowest WHC treatment (25% WHC), it was comparable to plants subjected to higher WHCs, particularly in plants grown under both 100% WHC (C100 and B100) and control plants of 75% WHC; C75.

Root profile

Root length and root length density measurements (*Fig. 12a, b*) indicate that biochar-treated plants of 50% WHC; B50 had the highest root length and root length density recorded, and is significantly higher (by 80.18%), than control of the same WHC; C50. Among the biochar-treated plants, plants subjected to 100% WHC; B100 recorded the lowest root length and root length density compared to other treatments, while biochar-treated plants of 75% and 25% WHC (B75 and B25) showed comparable results with each other. In contrast, control treatments were similar across all WHC (100%, 75%, 50% and 25% WHC). Overall, control plants had relatively lower root lengths and length density compared to biochar-treated plants of the same WHC, except for control plants grown under 100% WHC; C100.

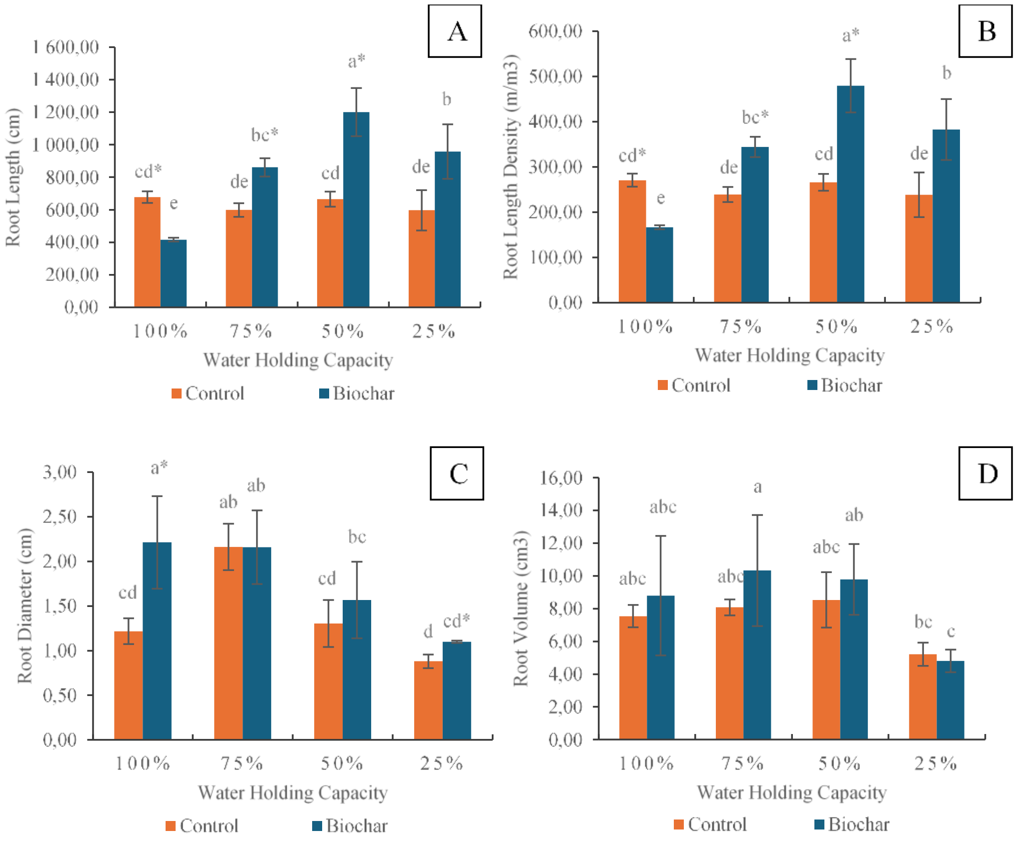

Figure 12. *Root profiles of R. tomentosa. (n=8). (a) Root length. (b) Root length density. (c) Root diameter. (d) Root volume. Vertical bars represent standard deviation. Different letters indicate significant differences at ($p \leq 0.05$) between treatments in week 12. Asterisks (*) indicate significant differences between control and biochar treatments of the same WHC at ($p \leq 0.05$)*

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In terms of root diameter (*Fig. 12c*), it was analysed that, at higher WHC treatments, particularly biochar-treated plants of 100% WHC; B100, and both plants of 75% WHC; C75 and B75 recorded significantly higher root diameter compared to plants subjected to lower WHC (50% and 25% WHC). Both plants grown under 75% WHC treatments; C75 and B75 were comparable to B100. Coincidentally, biochar-treated plants grown under 50% WHC; B50 were also comparable to both plants grown under 75% WHC treatments. Overall, root diameter was observed to decrease as WHC decreased. Within each treatment, both biochar and control treatments were observed to be comparable, except for control plants under 100% WHC; C100. Biochar-treated plants under 100% WHC possessed significantly higher root diameter (by 81.77%) than control plants of the same WHC; C100.

Root volume (*Fig. 12d*) of biochar-treated plants grown under 75% WHC; B75 yielded the highest root volume measured across all treatments. Although, the increase in root volume yielded by B75 was not statistically significant compared to other plants grown in high and lower soil WHC (100% and 50% WHC), and to control plants of the same WHC; C75. However, biochar-treated plants grown under 75% WHC were still significantly higher (by 98.29% and 114.75%) than plants grown under 25% WHC in both soil treatments; C25 and B25, respectively (*Fig. 13*).

Figure 13. *R. tomentosa post-harvest. Meter ruler for scale. (a) C100 (b) C75 (c) C50 (d) C25 (e) B100 (f) B75 (g) B50 (h) B25*

Parameter correlations

To determine the correlations between parameters measured during and post-experimental planting of *R. tomentosa*, a Pearson's correlation analysis was conducted, as outlined in *Table 5*. Analysis of results showed photosynthetic rate possessing a strong positive correlation with stomatal conductance ($r = 0.835$), relative water content ($r = 0.806$), and absolute water content ($r = 0.739$), indicating photosynthesis of *R. tomentosa* were increased as water availability and stomatal activity increased. Additionally, transpiration rate was also strongly positive correlated to stomatal conductance ($r = 0.997$), which validates the close physiological correlation between gaseous exchange and water loss due to transpiration, facilitated by the stomata.

Table 5. *Pearson's correlation coefficient physiology, morphology, root profile and biomass of R. tomentosa*

Parameters	**Physiological Response**										**Morphology**					**Biomass**		
	WHC	**Biochar Application**	**Relative Chlorophyll Content**	**Photosynthetic Rate**	**Stomatal Conductance**	**Transpiration Rate**	**Water Use Efficiency**	**Leaf Water Potential**	**Relative Water Content**	**Absolute Water Content**	**Plant Height**	**Root Length**	**Root Diameter**	**Root Volume**	**Root Length Density**	**Branch Biomass**	**Leaf Biomass**	**Root Biomass**
WHC	1																	
Biochar Application	-	1																
Relative Chlorophyll Content	-.570**	.256*	1															
Photosynthetic Rate	.811**	.443**	-.374**	1														
Stomatal Conductance	.798**	0.220	-.452**	.835**	1													
Transpiration Rate	.913**	0.003	-.483**	.887**	.997**	1												
Water Use Efficiency	-.845**	0.125	.536**	-.635**	-.693**	-.808**	1											
Leaf Water Potential	-.797**	0.084	.779**	-.762**	-.820**	-.899**	.719**	1										
Relative Water Content	.787**	0.202	-.586**	.758**	.761**	.794**	-.725**	-.749**	1									
Absolute Water Content	.762**	0.164	-.630**	.806**	.776**	.787**	-.797**	-.748**	.858**	1								
Plant Height	.572**	0.181	-.428**	.739**	.419**	.532**	-.602**	-.506**	.740**	.713**	1							
Root Length	-0.415	0.465	.736**	-0.201	-0.342	-0.287	0.533	0.593	-0.125	-0.576	0.148	1						
Root Diameter	.536*	0.305	-0.352	0.303	.516*	0.543	-0.506	-0.459	.644**	0.493	0.323	-0.149	1					
Root Volume	-0.084	-0.222	-0.142	-0.093	-0.165	0.342	0.359	0.002	-0.370	-0.410	-0.016	-0.108	0.003	1				
Root Length Density	-0.415	0.465	.736**	-0.201	-0.342	-0.287	0.533	0.593	-0.125	-0.576	0.148	1.000**	-0.149	-0.108	1			
Branch Biomass	.722**	-0.055	-.716**	.595*	.608**	.759**	-.765**	-.699**	.638**	0.488	.635**	-0.039	.555*	0.254	-0.039	1		
Leaf Biomass	0.349	0.226	-0.352	0.184	0.507	.627*	-0.319	-0.203	0.430	0.181	0.457	-0.033	.804**	0.128	-0.033	.599*	1	
Root Biomass	0.307	0.350	-0.113	0.352	0.305	0.462	-0.112	-0.300	0.363	0.157	0.235	0.100	0.507	0.078	0.100	.627**	.797**	1

Asterisk (*) indicate significance at ($p \leq 0.05$), Double asterisks (**) indicate signficance at ($p \leq 0.01$)

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Unexpectedly, relative chlorophyll content displayed significant moderate negative correlations with photosynthetic rate (r = -0.374), stomatal conductance (r = -0.483), and transpiration rate (r = -0.483) but was positively correlated with WUE (r = 0.536). This may suggest that *R. tomentosa* lowers its photosynthesis but also able to maintain higher pigment concentrations under more efficient water utilization, such as water stress. Morphologically, root diameter of *R. tomentosa* showed a significantly moderate positive correlation with photosynthetic rate (r = 0.516), relative water content (r = 0.644), and absolute water content (r = 0.631), suggesting that higher photosynthesis activity and water availability had driven better development of thicker roots, which can be beneficial for structural reinforcement and hydraulic conductivity. Analysis of results involving WHC showed that WHC had demonstrated significantly strong positive correlations with transpiration rate (r = 0.913), photosynthetic rate (r = 0.811), and stomatal conductance (r = 0.798), which indicates that overall metabolism increase of *R. tomentosa* was significantly dependent on the water availability in the soil Additionally, WHC had also showed significant positive correlations with several plant water status parameters, such as relative water content (r = 0.787), absolute water content (r = 0.762), and leaf water potential (r = 0.787), indicating that higher water availability in the soil contributed to better plant water content, as expected. Biochar application was also observed to have significantly positive moderate correlation with photosynthetic rate (r = 0.443) and significantly weak relative chlorophyll content (r = 0.256). This finding suggests that biochar application was able to positively influence photosynthesis activity and slightly improve chlorophyll production in *R. tomentosa*.

To examine the overall effects of WHC, biochar application along with their interactions, a multivariate analysis of variance (MANOVA) was performed on plant physiology, morphology and biomass. Hotelling-Lawley's test (*Table 6*) indicated that both biochar (λ = 46.452, p < 0.0001) and WHC (λ = 412.934, p < 0.0001) effect were significant. Furthermore, the interaction effect of WHC and biochar application was significantly observed (λ = 100.645, p < 0.0001), which suggests that the biochar effect on measured parameters of physiology, morphology and biomass was also dependent on soil WHC.

Table 6. *Multivariate Analysis of Variance (MANOVA) for the overall effects of WHC and biochar towards parameters measured*

	WHC	**Biochar Application**	**WHC*Biochar Application**
Lambda	412.934	46.452	100.645
F (Observed values)	341.244	119.034	83.172
DF1	48	16	48
DF2	119	41	119
F (Critical value)	1.464	1.897	1.464
p-value	< 0.0001	< 0.0001	< 0.0001

For further detailed analysis, a two-way Analysis of Variance (ANOVA) was conducted to assess the overall effects of WHC, biochar application and interaction between WHC and biochar application (WHC x biochar application) on each individual parameters measured in this study, as outlined in *Table 7*. Based on the analysis, WHC had a significant effect on all physiological parameters of *R. tomentosa* (all p < 0.001), which indicates that soil water availability has significant influence on the metabolic processes such as photosynthetic rate and relative chlorophyll content, and plant water status. Biochar applications had also significantly improved several physiological parameters in this study. Notably, biochar application had enhanced photosynthetic rate (p < 0.001), WUE (p < 0.001), leaf relative water content (p = 0.001), and leaf absolute water content (p < 0.001). This may suggest that biochar properties had improved photosynthesis of *R. tomentosa*, through an improvement in soil available water and optimization of water utilization in plants.

Table 7. *Two-way Analysis of Variance (ANOVA) for the effects of WHC and biochar applications towards plant physiology, morphology and biomass of R. tomentosa*

	WHC				**Biochar Application**				**WHC x Biochar Application**			
	F	***df***	***p***	***Sig.***	***F***	***df***	***p***	***Sig.***	***F***	***df***	***p***	***Sig.***
Plant Physiology												
Relative Chlorophyll Content	11.619	3	<0.001	*	6.931	1	0.011	*	0.623	3	0.603	n.s.
Photosynthetic Rate	89.161	3	<0.001	*	68.489	1	<0.001	*	6.434	3	0.001	*
Stomatal Conductance	35.298	3	<0.001	*	6.192	1	0.017	*	1.787	3	0.164	n.s.
Transpiration Rate	160.720	3	<0.001	*	2.180	1	0.150	n.s.	12.051	3	<0.001	*
Water Use Efficiency	137.081	3	<0.001	*	21.240	1	<0.001	*	10.025	3	<0.001	*
Leaf Water Potential	17.636	3	<0.001	*	0.422	1	0.522	n.s.	0.716	3	0.552	n.s.
Leaf Relative Water Content	55.439	3	<0.001	*	13.566	1	0.001	*	2.520	3	0.072	n.s.
Leaf Absolute Water Content	175.167	3	<0.001	*	27.465	1	<0.001	*	2.385	3	0.097	n.s.
Plant Morphology												
Plant Height Increment	15.731	3	<0.001	*	3.170	1	0.081	n.s.	0.245	3	0.865	n.s.
Root Length	10.930	3	0.003	*	21.746	1	0.002	*	12.680	3	0.002	*
Root Diameter	13.521	3	0.001	*	7.900	1	0.017	*	2.755	3	0.093	n.s.
Root Volume	0.649	3	0.598	n.s.	0.543	1	0.475	n.s.	0.597	3	0.629	n.s.
Root Length Density	10.930	3	0.003	*	21.746	1	0.002	*	12.680	3	0.002	*
Biomass												
Branch	6.390	3	0.008	*	0.110	1	0.746	n.s.	0.328	3	0.805	n.s.
Leaf	13.728	3	0.001	*	5.554	1	0.043	*	2.339	3	0.142	n.s.
Root	9.166	3	0.002	*	3.605	1	0.080	n.s.	1.218	3	0.343	n.s

Asterisk (*) indicate that the parameter was significant at ($p \leq 0.05$). n.s. indicate that the parameter is not significant at ($p \leq 0.05$)

Although, there were no significant effect of biochar applications for transpiration rate (p = 0.150) and leaf water potential (p = 0.522). However, significant interaction effects of WHC and biochar applications were observed on transpiration rate (p < 0.001), besides photosynthetic rate (p = 0.001), and WUE (p < 0.001), which suggests that transpiration rate were highly affected by WHC and in its interaction with biochar, instead of from the sole effect of biochar. Additionally, no further significant interaction effects were observed for other physiological parameters; relative chlorophyll content (p = 0.603), stomatal conductance (p = 0.164), leaf water potential (p = 0.552), leaf relative water content (p = 0.0.72) and leaf absolute water content (p = 0.097). In terms of plant morphology, WHC significantly affected plant height increment (p < 0.001), root length (p = 0.003), root diameter (p = 0.001), and root length density (p = 0.003), but was not significant towards root volume (p = 0.598). Effects of biochar application were also observed significant, on root length (p = 0.002), root diameter (p = 0.017), and root length density (p = 0.002), but was not significant on the impact of plant height increment (p = 0.081) and root volume (p = 0.475). Notably, interaction effects of WHC and biochar were only significant for root length and root length density (all p = 0.002), which suggests that plant height increment and root diameter of *R. tomentosa* were heavily influenced by individualistic effect (WHC), while root lengths were influenced by both individual and collective WHC and biochar applications. Unexpectedly, root volume was not significantly influenced by any treatments subjected in this study. In terms of biomass of *R. tomentosa*, significant effects of WHC were observed on all three (3) biomass parameters; branch biomass (p = 0.008), leaf biomass (p < 0.001), and root biomass (p = 0.002). On the contrary, biochar application only was significant on leaf biomass (p = 0.002), while there was no significant influence on branch (p = 0.746) and root biomass (p = 0.080). Notably, interaction of WHC and biochar applications were not significant for any of the biomass parameters; branch (p = 0.805), leaf (p = 0.142) and root biomass (p = 0.343). This suggests that all biomass parameters studied were significantly affected by WHC while biochar application only had influence on the leaf biomass of *R. tomentosa*, and when combined, its effects were overall insignificant.

Discussion

Relative chlorophyll content

Changes in chlorophyll levels serve as early indicators of plant stress, as chlorophyll is the primary pigment responsible for photosynthesis in plants (Shao et al., 2024). Water stress is known to cause changes in photosynthetic properties, such as damage to the Photosystem II reaction centre. This damage disrupts electron transport function, increased photoinhibition and reduced efficiency of primary light reaction, which in tandem, leads to changes in chlorophyll fluorescence parameters measured (Wang et al., 2024). The utilization of SPAD meter to measure chlorophyll content also relies on the abundance of nitrogen and potassium within the plant, as these nutrients aid synthesis of chlorophyll (Uysal, 2018). Moreover, reduced chlorophyll content could be an indicator of nitrogen deficiency, as there is a close relationship between the levels of nitrogen and the pigmentation production within the plant (Joko et al., 2024). According to data obtained in *Figure 2*, lower WHC, 50% and 25% WHC achieved significantly higher SPAD value, which indicates that relative chlorophyll content within the leaves measured were higher opposed to “waterlogged” (100% WHC) and “control” (75% WHC) treatments. These results correspond to another study by Mulugeta et al. (2023) on *Ocium* sp., another type of shrub plant, whereby plants under lower soil water capacity (SWC)%, similar to the concept of WHC%, under drought stress had yielded significantly higher SPAD values. This could be attributed to an increase in shrinkage and leaf wilting, instead of chlorophyll content, which could also result in a much richer green colour in comparison to turgid and expanded leaves of plants under higher WHC treatments (Mulugeta et al., 2023).

When examining the effects of biochar on SPAD values, the analysed data showed no significant differences across treatments, except for B75 compared to its control, C75. This suggests that while biochar had a minimal an effect on improving relative chlorophyll content, its impact was not consistent across all treatments. Theoretically, addition of biochar into the soil should enhance SPAD values, by improving nutrient availability as described by Rusli et al. (2021), who reported significant improvements in chlorophyll content with biochar application. However, in this study, under severe water stress, biochar treatment did not significantly enhance *R. tomentosa* SPAD values, suggesting that water stress may have negated its potential benefits Consequently, the advantage of biochar application over the control treatments could not be confirmed through SPAD analysis alone, particularly under mild and severe water stress conditions. Overall, biochar showed a limited impact on SPAD values, with only B75 plants exhibiting a minor improvement over its control (C75), while other treatments did not show significant differences.

Physiological responses

Photosynthetic rate, transpiration rate and stomatal conductance

Photosynthesis plays a vital part of a plant's process to thrive. However, generally during water stress, both plant growth and productivity resulting from photosynthesis activities are expected to be significantly affected, which can impact its survivability, which could eventually lead to mortality. During water stress, rates of photosynthesis will be affected due to reduced availability of CO_2, resulting from stomatal closure (Osakabe et al., 2014). For the average photosynthetic rate of *R. tomentosa* in different treatments, it can be deduced that each treatment at different WHC were expected to be decrease accordingly. However, biochar treatment benefits were more prominent for the photosynthetic rate in each water levels. At higher WHC, it was observed that B100 had significantly better photosynthetic rates compared to its control, C100, albeit being waterlogged condition, which may be attributed to better aeration through better soil porosity, better nutrient uptake into the plants which may lead to better photosynthetic activities by biochar addition (Shao et al., 2024). Under mild and severe water stress (50% and 25% WHC), biochar treatments were comparable to 75% WHC and had significantly improved photosynthetic rate compared to its control of the same tier. This can be attributed through biochar's ability to improve soil water retention (Shao et al., 2024) which allows water to be held better and are readily available for the plants. An increase in the surface area contributed by biochar allows it to hold much more water per volume of soil, whilst allowing water retention through biochar's porous structure (Kabir et al., 2023). Furthermore, biochar had also been reported to allow an increase in WHC of soil for up to 30%, which indicate that, for similar WHC of both treatments, biochar-treated soil may hold more moisture content compared to control soils, which is dependent on the type of biochar used (Kabir et al., 2023).

For stomatal conductance, almost similar trend was observed with photosynthetic rate of the plants, as both aspects are closely related with each other. However, at high WHC treatments, there were no observed significant differences between B100, C100 and B75. although B100 had the highest average stomatal conductance measured amongst the three treatments, whilst C100 and B75 had similar values at the end of the experimental planting. Stomatal conductance is the measure of stomatal opening and considered an indirect indicator towards plant water status, by governing gaseous diffusion between leaf and the atmosphere, regulating CO_2 assimilation, water loss and cooling through evaporative means (Giménez et al., 2013; Faralli et al., 2019). Under mild and immense water stress, photosynthesis of plants will eventually experience a drop, through partial closure of stomata to reduce significant water loss due to transpiration (Diatta et al., 2021). Through data gathered as aforementioned, it can be observed that higher WHC treatments experienced reduced impacts of photosynthesis and plant-water content regulation through stomata, which is reflected in much higher values compared to mild and severe water stressed plants. At lower WHC levels, stomatal conductance was also not significantly different with each other, which could indicate that, at certain soil WHC levels under water stress, especially at severe water stress (25% WHC), *R. tomentosa* could regulate its stomatal conductance similar to plants under mild water stress (50% WHC), showing potential in efficient control in water preservation. Even so, biochar treatments still showed slightly better average measurements, specifically B25, potentially attributed by better soil properties, such as better water retention and overall available moisture within the soil itself, through the porous structure and larger surface area that could contain water, contributed by the biochar.

In terms of transpiration rate, there were slight differences observed when compared to photosynthetic rate and stomatal conductance. Generally, measurements of transpiration rate were more stable across 6 points of measurements during 12 weeks of treatment, whereas photosynthetic rate and stomatal conductance experiences rises and significant drops. This could be attributed to photosynthetic rate and stomatal conductance is dependent on much more factors, such as nutrients and CO_2 as compared evaporation and water content of the plants for transpiration rate measurements. At severe water stress, transpiration rate was still measured to be comparable to each other, which may indicate that at certain point, *R. tomentosa* is able to limit transpiration, whilst allowing the plant to still survive at a critical condition, as aforementioned. When comparing between control and biochar-treated plants, significant impact on the positive difference can be observed on B25, as opposed to control; C25, which can be attributed to better water retention and overall water availability contributed by biochar under 25% WHC. Based on the results, it was also discovered that C50 had higher transpiration rate compared to B50 (by 51.37%) at the end of experimental planting week, while previously measured transpiration rates were almost comparable to each other. This could be attributed to various factors, such as temperature, weather and humidity at the time of measurement, which can affect transpiration rates variability, which is one of the more volatile aspects to measure. To better understand and precisely study this aspect, a controlled environment would be preferable, although it does not reflect the actual conditions the plant would experience in nature.

Water use efficiency (WUE)

WUE can be defined as the ratio of carbon assimilation (or carbon storage) process and water release commonly used for input/output of productivity measurement, in this case, for photosynthesis (Stanhill, 1986; Halli et al., 2022; Fatichi et al., 2023). This can be calculated by the ratio of photosynthetic rate that takes place and the transpiration rate during the process. From measurement obtained, B25 achieved the highest average WUE across all treatments and was significantly different compared to its control, C25. This may be attributed to a moderately average photosynthetic rate, yet very low transpiration rate exhibited by the treatment which could indicate that productivity of the plant is considerable even when hindered by the lack of available water supply from the biochar-infused soil. This finding coincides research findings of Batool et al. (2015), whereby other plant species, such as *A. esculentus* L. Moench had shown improved WUE under water stress conditions, through a decrease in transpiration rate, yet an improved photosynthetic rate through the addition of biochar.

Leaf relative water content and absolute water content

Leaf relative water content (RWC) is considered as a direct indicator of the water status for a plant, whereby RWC is a measurement for the water content of a plant tissue, in this case, the leaf relative to its maximum water capacity during full saturation (Giménez et al., 2013). Leaf RWC measured remained relatively consistent every measurement across week 3, 6 and 12 of experimental planting. Mild and severe WHC treatments were expected have a significantly lower RWC compared to higher water levels. However, although, 50% and 25% WHC was still significantly measured lower than 100% and 75% WHC, on certain weeks measurements were comparable to higher WHC treatments. Besides environmental factors, this may indicate that *R. tomentosa* could be efficient in managing water content within cells of the plants, as indicated through transpiration rate results previously. This allows *R. tomentosa* to be able to potentially maintain its turgidity and maintain productivity through its leaf water content, when facing drought conditions. At both higher and lower WHCs, there were no significant indicators of biochar improvement towards RWC. Nevertheless, biochar treatments across most of WHC were still slightly higher compared to its control counterparts, almost similar, in theory, to results obtained by Hoang et al. (2021) that tested different percentage of biochar application towards *E. asperula* under water stress.

AWC is much more uncommon measurement of studying water content within an organ of the plant yet still bear an importance of plant water status indicator as with RWC. As both RWC and AWC measurements were using invasive techniques, of harvesting and floating leaves on distilled water to measure, AWC measurements were only taken once, during week 12, to identify current plant water level content at the near end of the experimental planting in order to prevent further loss of leaves. This is due to plants under mild and severe water stress does not yield new expanded leaves often compared to higher water level treatments. AWC measurement of treatments indicate that at higher WHC levels, 100% WHC, there were no significant differences observed, while at 75% WHC, biochar is significantly higher than its control counterpart. At lower WHC levels, similar occurrence also could be observed, where C50 and B50 had no significant differences, while B25 is significantly higher than C25. As biochar provide the benefit of allowing water to be retained better within amended soil, through better soil water retention, it can be tied to a better leaf AWC, where available water is more accessible, and plants are able to uptake water for storage or for its metabolism processes, such as photosynthesis.

Leaf water potential (LWP)

Leaf water potential (LWP) is also an important physiological characteristic to study plant tolerance to water scarcity (Reddy et al., 2021). Plants that experience water/drought stress will restrict water supply, thus reducing leaf water content and its water potential in order to reduce water loss to transpiration and photosynthesis process, subsequently reducing turgor of the leaves, causing wilting and leaf rolling characteristic to be prominent within severe water stress plants in this study (Rani et al., 2019; Reddy et al., 2021). Based on data obtained, it is expected as per hypothesis that the leaf water potential would significantly decrease the lower the WHC levels of the treatment. Similarly, at lower WHC levels, leaf water potential measurement was closely related, tying close correlations to transpiration rates, leaf RWC and AWC. In all treatments, except for B50 and B25, measured to be slightly higher than its control. However, none of the lower WHC treatments (50% and 25% WHC) were comparable to water level treatments that are a tier-higher and above (100% and 50% WHC). Although, LWP of B25 had similar measurements of both 50% WHC treatments, which could indicate that there may be a slight improvement due to addition of biochar, under severe water stress.

Plant height and biomass

Plant height increments of the treatments that was observed at the first 2 to 4 weeks of experimental planting indicate that C100 achieved the highest immediate plant height increment, only second to B100 and followed by plants in subsequent decreasing water level treatments. However, average plant growth increment at the end of the study showed that B75 achieved the highest average increase instead, compared to plants under 100% WHC treatments. This may indicate that plants under 100% WHC, in both control (C100) and biochar (B100) treatment, were waterlogged and had stunted the rapid growth of *R. tomentosa* especially after the first 2 to 4 weeks of experimental planting. Similarly, previous research on soybeans by Adegoye et al. (2023) had indicated that waterlogged plants had experienced significantly decreased physiology, one of which is plant height, compared to other plants. Although, at lower WHC level, plant height increment of biochar treatments, especially B25 were comparable to plants under 50% WHC (C50 and B50), which may indicate that biochar could support the increase in plant growth, even under immense water stress, compared to control. This could be attributed to better plant nutrient retention such as nitrogen through soil cation exchange capacity by the negatively charged functional groups that are abundant on the surface of biochar particles and soil moisture retention by biochar soil amendment (Rani et al., 2019; McLennon et al., 2020; Kabir et al., 2023).

Biomass of the plant is highly dependent on the plant productivity, such as plant height increment, for branch biomass together with leaf and root biomass. Results had indicated that branch biomass to scale similarly together with results obtained for plant height increment, where B75 achieved the highest average branch biomass compared to its control, and across all treatments. However, it should be noted that under experimental planting, less branching had occurred to all plants, which could be attributed to root confinement in small containers such as pot used in this study, which substantially reduced shoot growth (Hameed et al., 1987). In terms of leaf biomass, it should also be anticipated that water stress treatments would lead to a reduction of fully expanded leaves and new leaves growth while old leaves fall off due to natural factors. This can be observed significantly at lower WHC levels, 50% and 25% WHC treatments that had the lowest leaf biomass measured, due to lack of new leaves. On the other hand, 75% WHC, particularly biochar treatment (B75) achieved the highest leaf biomass compared to control and across all treatments. Due to being waterlogged status, 100% WHC, total leaf biomass measured at the end of the experiment was significantly lower, potentially attributed to significant decline in soil oxygen concentrations and nutrient availability which subsequently impact physiology and productivity of the plants such as photosynthesis, similar to waterlog soybean research by Adegoye et al. (2023). Root biomass also exhibited relatively similar results, where B75 achieved the highest root biomass. However, both C50 and B50 achieved comparable results to B75, which may be the adaptation effect towards water stress by scavenging different parts of the soil for moisture.

Root profile

Generally, *R. tomentosa* that were treated with biochar observed to have the highest average root length and root length density, particularly under 75%, 50% and 25% WHCs, where biochar treatment was significantly higher than its control counterpart. Across all treatments, B50 achieved the highest root length and density, only second to B25. This result was similar of Rusli et al. (2021), which could indicate that macronutrients contained in biochar had an influence on enhancing root growth. Additionally, better soil porosity, soil water retention and nutrient holding capacity of biochar may aid the elongation and growth of roots within biochar-treated *R. tomentosa* by providing better confinement for roots to grow, available access to water and nutrients to the plant, respectively (Azab and Hegazy, 2020; Gaber et al., 2024). In terms of root diameter and root volume, biochar treatments across every WHC were comparable to its control. However, for root diameter, B100 achieved the highest difference to control, which could indicate, in order to compensate for oversaturated soil structure by having better anchor towards the soil. For mild and severe water stress treatments, biochar treatment had slightly higher root diameter and volume, which could also aid in providing improved stability and anchor force within a water-deficient soil, compared to control. Nonetheless, deficient in root profiles observed in this study may be the repercussion of experimental planting, being confined in plant pots that could be less ideal, especially for root growth studies due to plants that are constricted in limited soil volume compared to field conditions (Azab and Hegazy, 2020). Thus, it could be hypothesized that better root growth, in terms of length density, diameter and volume will be expected for *R. tomentosa* under better conditions, such as natural field planting.

Correlations of parameters

According to Pearson's correlations analysis in *Table 5*, photosynthetic rate of *R. tomentosa* exhibited a strong positive correlation, with stomatal conductance ($r = 0.835$), relative water content ($r = 0.806$), and absolute water content ($r = 0.739$). Higher photosynthetic rate of *R. tomentosa*, as observed in this study, may be the result of greater influx of carbon dioxide (CO_2) through gaseous exchange facilitated by the higher stomatal conductance, leading to an increase in overall photosynthesis. Additionally, the significant correlation between transpiration rate and stomatal conductance ($r = 0.997$), which suggests that the stomata was also effective in regulating transpiration, given the fact that stomatal conductance being responsible for controlling water vapour diffusion from mesophyll interstitial cells to the surface of stomata on the leaf (Zhu et al., 2022). It was only unexpectedly observed that relative chlorophyll content was negatively correlated with photosynthetic rate ($r = -0.374$), given the fact that chlorophyll is an important pigment associated in photosynthesis process. However, this may suggest that although higher pigment concentrations were observed, *R. tomentosa* was able to adapt, by reducing photosynthesis activity as a response to unfavourable conditions, such as water stress. This can also be supported by the significant positive correlation of relative chlorophyll content and WUE ($r = 0.536$), in which suggests better water utilization had improved chlorophyll content of *R. tomentosa*. For root profiles, root diameter in *R. tomentosa* showed moderate positive correlations with photosynthetic rate (r = 0.516), relative water content (r = 0.644) and absolute water content (p = 0.631), which may suggest that photosynthesis, aided with plant water status represented by both relative and absolute water content, were significant on increasing root diameter, to achieve better water and nutrient uptake besides improved structural stability. Notably, as biochar application moderately correlates with photosynthesis (r = 0.443) and weak correlation with chlorophyll content (r = 0.256), it may be the indication that biochar was also able to enhance photosynthesis and pigmentation. However, overall, there were no significant correlations of biochar improving plant water status parameters, such as transpiration rates and water use efficiency.

A multivariate ANOVA (MANOVA) analysis was conducted, to assess the overall effectiveness of WHC and biochar interaction affects. However, as observed in *Table 6*, the individual effects of WHC and biochar, together with interaction effects were all observed to be significant (p < 0.0001), which is a positive indicator, especially for combined effects of WHC and biochar application were overall significant and had an effect in this study. However, the significance of the interaction effects on each parameter could not be observed and validated through only general MANOVA. Thus, A two-way ANOVA analysis was conducted, to examine the effects of soil WHC and biochar application on each physiology, morphology, and biomass parameter of *R. tomentosa* in this study. Results in *Table 7* showed that WHC had significantly influenced all physiological traits of the species, including photosynthetic rate (p < 0.001), transpiration rate (p < 0.001) and stomatal conductance (p = <0.001). Additionally, WHC also had a strong effect on plant morphology, with significant effects on plant height increment (p < 0.001) root length (p = 0.003), root diameter (p = 0.001), and root length density (p = 0.003). Biomass production was also significantly affected, in branch (p = 0.008), leaf (p = 0.001) and root biomass (p = 0.002). Biochar application also independently influenced similar parameters to WHC, except for transpiration rate (p = 0.150) and leaf water potential (0.522); plant height increment (p = 0.081); together with branch biomass (p = 0.746) and root biomass (p = 0.080). Based on this analysis, biochar application did significantly affect the relative chlorophyll content (p = 0.011), but it was observed in the SPAD value measurements that biochar only contributed limited chlorophyll improvement in *R. tomentosa.* This may suggest that although the improvement in chlorophyll content was limited, it was significant to indicate that it was a measurable physiological increase, in response to biochar application, possibly contributed by enhanced nutrient availability and water stress mitigation. The interaction effect of biochar with WHC was only significant in a few parameters, such as photosynthetic rate (p = 0.001), transpiration rate (p < 0.001), WUE (p < 0.001), root length (p = 0.002) and associated root length density (p = 0.002). Zoghi *et al.* (2019) also reported multiple non-significant “biochar x water stress” interaction in *Quercus castaneifolia*, except for several morphological traits, such as height. However, in this study, *R. tomentosa* plant height increment was not affected by the interaction effect and sole biochar application, indicating WHC had significantly strong effect (p < 0.001) in influencing overall plant height increment. Notably, WHC-biochar interaction had significance on root length and root length density. This could be the result of biochar application had likely enhanced root growth of *R. tomentosa*, through improved soil water retention, and nutrient availability at lower WHCs, being under water stress (Zhang et al., 2020).

Conclusion

The finding of this study indicates that biochar, specifically made from palm kernel, had several significant improvements towards *R. tomentosa* including plants that are under water stress from mild and severe levels of WHC. Application of biochar were able to slightly improve photosynthetic efficiency through photosynthetic rate, stomatal conductance transpiration rate and WUE, besides aiding in plant growth and improved soil WHC, measured through RWC and root profiles. This was achieved through biochar properties that have been proven in previous literatures, such as having better soil water retention through an increase in soil porosity by the porous structure of biochar, that are able to fill water by increase in surface area, which subsequently increase soil WHC and indirectly promoting nutrient retention and uptake, through an improved soil cation exchange capacity (CEC) by the negative charged functional groups on surface of biochar. Although the interaction between WHC and biochar application were not statistically significant for most parameters, in physiology, morphology and biomass, biochar application still had contributed to improvements in several key parameters, such as photosynthetic rate ($p < 0.001$) and WUE ($p < 0.001$) of *R. tomentosa*. Importantly, biochar application had enhanced root morphology, including root length ($p = 0.002$), root diameter ($p = 0.017$), and root length density ($p = 0.002$), which were also significantly influenced by the interaction effect of both WHC and biochar application, except root diameter ($p = 0.093$). Additionally, leaf dry biomass showed a positive response to biochar treatment ($p = 0.043$), which suggests potential benefit attributed by biochar in supporting plant resilience under different soil conditions, especially in water deficit. While WHC played an overall dominant role in plant physiological response, the inclusion of biochar may still offer several advantages in improving plant structure and resource uptake through soil conditioning with biochar, under varying water availability, especially in rough, field conditions. This study provides an early insight into potentially adapting *R. tomentosa* and also PK biochar in order to mitigate the detrimental effects of climate change, specifically drought towards maintaining the structural integrity of soil. Based on the data obtained, PK biochar-treated soil had provided advantages across all WHC treatments and has been observed to have benefits for several WHC treatments, to optimize physiological attributes during normal conditions and during water deficit conditions. Although *R. tomentosa* also had survived on non-biochar amended soil, but through the data analysis obtained in this study, the reduction in all physiological response measurements including root profile may not be substantial for the species to survive long term, especially in rough conditions such as on slope. Further studies would be suggested to further examine the full potential of *R. tomentosa* under water stress in field conditions, and explore other potential soil amendments and levels, particularly, biochar sources other than palm kernel biochar, to further improve the ability of *R. tomentosa* to survive water-deficient environments.

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REFERENCES

[1] Adegoye, G., Olorunwa, O., Alsajri, F., Walne, C., Wijewandana, C., Kethireddy, S., Reddy, K., Reddy, K. (2023): Waterlogging Effects on Soybean Physiology and Hyperspectral Reflectance during the Reproductive Stage. – Agriculture 13: 844. https://doi.org/10.3390/agriculture13040844.

[2] Azab, E., Hegazy, A. K. (2020): Monitoring the Efficiency of *Rhazya stricta* L. Plants in Phytoremediation of Heavy Metal-Contaminated Soil. – Plants 9(9): 1057. https://www.mdpi.com/2223-7747/9/9/1057.

[3] Batool, A., Taj, S., Rashid, A., Khalid, A., Qadeer, S., Saleem, A. R., Ghufran, M. A. (2015): Potential of soil amendments (Biochar and Gypsum) in increasing water use efficiency of *Abelmoschus esculentus* L. Moench [Original Research]. – Frontiers in Plant Science 6: 733. https://doi.org/10.3389/fpls.2015.00733.

[4] Chen, H., Wu, Y.-C., Cheng, C.-C., Teng, C.-Y. (2023): Effect of climate change-induced water-deficit stress on long-term rice yield. – PLOS ONE 18(4): e0284290. https://doi.org/10.1371/journal.pone.0284290.

[5] Csurhes, S., Hankamer, C. (2016): Invasive plant risk assessment: Ceylon hill cherry (downy rose myrtle) *Rhodomyrtus tomentosa*. – Queensland Government. https://www.daf.qld.gov.au/__data/assets/pdf_file/0005/71942/IPA-Ceylon-Hill-Cherry-Risk-Assessment.pdf.

[6] Diatta, A. A., Min, D., Jagadish, S. V. K. (2021): Chapter Two - Drought stress responses in non-transgenic and transgenic alfalfa: Current status and future research directions. – In: Sparks, D. L. (ed.) Advances in Agronomy 170: 35-100. Academic Press. https://doi.org/https://doi.org/10.1016/bs.agron.2021.06.002.

[7] FAO. (2023): Standard operating procedure for soil moisture content by gravimetric method. – Food and Agriculture Organization of United Nations. Retrieved 28 January from https://openknowledge.fao.org/server/api/core/bitstreams/e8811ce2-af62-470f-90fb-71b1da2d00c0/content.

[8] Faralli, M., Matthews, J., Lawson, T. (2019): Exploiting natural variation and genetic manipulation of stomatal conductance for crop improvement. – Current Opinion in Plant Biology 49: 1-7. https://doi.org/https://doi.org/10.1016/j.pbi.2019.01.003.

[9] Fariñas, M. D., Jimenez-Carretero, D., Sancho-Knapik, D., Peguero-Pina, J. J., Gil-Pelegrín, E., Gómez Álvarez-Arenas, T. (2019): Instantaneous and non-destructive relative water content estimation from deep learning applied to resonant ultrasonic spectra of plant leaves. – Plant Methods 15(1): 128. https://doi.org/10.1186/s13007-019-0511-z.

[10] Fatichi, S., Paschalis, A., Bonetti, S., Manoli, G., Pappas, C. (2023): Water use efficiency: A review of spatial and temporal variability. – In: Goss, M. J., Oliver, M. (eds.) Encyclopedia of Soils in the Environment (Second Edition). Academic Press, pp. 527-542. https://doi.org/https://doi.org/10.1016/B978-0-12-822974-3.00166-X.

[11] Gaber, M., Abdel Nasser, G., Hussein, A. (2024): Positive Effects of Biochar on Pepper Growth, Yield, and Root Distribution under Water Stress Conditions. – Alexandria Journal of Soil and Water Sciences 8(2): 57-75. https://doi.org/10.21608/ajsws.2024.256889.1013.

[12] Giménez, C., Gallardo, M., Thompson, R. B. (2013): Plant–Water Relations. – In Reference Module in Earth Systems and Environmental Sciences. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-409548-9.05257-X.

[13] Halli, H. M., Hatti, V., Gupta, G., Raghavendra, M., Meena, M. P., Gouda, R. (2022): Scientific approaches for water resources management in developing countries. – In: Srivastav, A. L., Madhav, S., Bhardwaj, A. K., Valsami-Jones, E. (eds.) Current Directions in Water Scarcity Research. Vol. 6, Chapter 7, pp. 129-147. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-91838-1.00017-8.

[14] Hameed, M. A., Reid, J. B., Rowe, R. N. (1987): Root Confinement and its Effects on the Water Relations, Growth and Assimilate Partitioning of Tomato (*Lycopersicon esculentum*

Mill). – Annals of Botany 59(6): 685-692. https://doi.org/10.1093/oxfordjournals.aob.a087367.

[15] Hatfield, J. L., Dold, C. (2019): Water-Use Efficiency: Advances and Challenges in a Changing Climate [Review]. – Frontiers in Plant Science 10. https://doi.org/10.3389/fpls.2019.00103.

[16] Hoang, T.-L.-H., Jang, D.-C., Nguyen, Q.-T., Na, W.-H., Kim, I.-S., Vu, N.-T. (2021): Biochar-Improved Growth and Physiology of *Ehretia asperula* under Water-Deficit Condition. – Applied Sciences 11(22): 10685. https://www.mdpi.com/2076-3417/11/22/10685.

[17] Ibrahim, H., Al-Wabel, M., Usman, A., Alomran, A. (2013): Effect of Conocarpus Biochar Application on the Hydraulic Properties of a Sandy Loam Soil. – Soil Science 178: 165-173. https://doi.org/10.1097/SS.0b013e3182979eac.

[18] Johnson, J. B. (2023): Rapid Prediction of Leaf Water Content in Eucalypt Leaves Using a Handheld NIRS Instrument. – Eng 4(2): 1198-1209. https://www.mdpi.com/2673-4117/4/2/70.

[19] Joko, L. B., Eiasu, B. K., Ngwenya, S. M. (2024): The Impact of Nitrogen Levels on the Growth, Development, and Yield of Orange-fleshed Sweet Potatoes (*Ipomoea batatas* L.). – HortScience 59(10): 1520-1523. https://doi.org/10.21273/hortsci17996-24.

[20] Kabir, E., Kim, K.-H., Kwon, E. E. (2023): Biochar as a tool for the improvement of soil and environment [Review]. – Frontiers in Environmental Science 11. https://doi.org/10.3389/fenvs.2023.1324533.

[21] Kim, H., Seomun, S., Yoon, Y., Jang, G. (2021): Jasmonic Acid in Plant Abiotic Stress Tolerance and Interaction with Abscisic Acid. – Agronomy 11(9): 1886. https://www.mdpi.com/2073-4395/11/9/1886.

[22] Kong, S.-H., Loh, S. K., Bachmann, R. T., Zainal, H., Cheong, K. Y. (2019): Palm kernel shell biochar production, characteristics and carbon sequestration potential. – J. Oil Palm Res 31(3): 508-520.

[23] Lloret, F., Escudero, A., Iriondo, J. M., Martínez-Vilalta, J., Valladares, F. (2012): Extreme climatic events and vegetation: the role of stabilizing processes. – Global Change Biology 18(3): 797-805. https://doi.org/https://doi.org/10.1111/j.1365-2486.2011.02624.x.

[24] Mansoor, S., Kour, N., Manhas, S., Zahid, S., Wani, O. A., Sharma, V., Wijaya, L., Alyemeni, M. N., Alsahli, A. A., El-Serehy, H. A., Paray, B. A., Ahmad, P. (2021): Biochar as a tool for effective management of drought and heavy metal toxicity. – Chemosphere 271: 129458. https://doi.org/10.1016/j.chemosphere.2020.129458.

[25] McLennon, E., Solomon, J. K. Q., Neupane, D., Davison, J. (2020): Biochar and nitrogen application rates effect on phosphorus removal from a mixed grass sward irrigated with reclaimed wastewater. – Sci Total Environ 715: 137012. https://doi.org/10.1016/j.scitotenv.2020.137012.

[26] Mickovski, S. B., van Beek, L. P. H. (2007): Decision support systems in eco-engineering: the case of the SDSS. – In: Eco- and Ground Bio-Engineering: The Use of Vegetation to Improve Slope Stability, pp. 361-367. Springer.

[27] Mishra, N., Jiang, C., Chen, L., Paul, A., Chatterjee, A., Shen, G. (2023): Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms [Review]. – Frontiers in Plant Science 14. https://doi.org/10.3389/fpls.2023.1110622.

[28] Mohd Roseli, A. N., Tsan, F. Y., Fauzi, R. (2010): Growth inhibition of *Syzygium campanulatum* Korth. for container planting by the application of uniconazole. – Pertanika Journal of Tropical Agricultural Science 33: 1-6.

[29] Mulugeta, S. M., Sárosi, S., Radácsi, P. (2023): Physio-morphological trait and bioactive constituents of Ocimum species under drought stress. – Industrial Crops and Products 205: 117545. https://doi.org/https://doi.org/10.1016/j.indcrop.2023.117545.

[30] Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.-S. P. (2014): Response of plants to water stress [Mini Review]. – Frontiers in Plant Science 5. https://doi.org/10.3389/fpls.2014.00086.

[31] Piñuela, Y., Alday, J. G., Oliach, D., Bolaño, F., Colinas, C., Bonet, J. A. (2020): Use of Inoculator Bacteria to Promote *Tuber melanosporum* Root Colonization and Growth on *Quercus faginea* Saplings. – Forests 11(8): 792. https://www.mdpi.com/1999-4907/11/8/792.

[32] Rani, P., Rai, S., Prasad, S., Singh, R., Nayak, H. (2019): Biochar: Moisture stress mitigation. – Journal of Pharmacognosy and Phytochemistry SP5: 299-307.

[33] Reddy, Y. A. N., Reddy, Y. N. P., Ramya, V., Suma, L. S., Reddy, A. B. N., Krishna, S. S. (2021): Drought adaptation: Approaches for crop improvement. – In: Singh, M., Sood, S. (eds.) Millets and Pseudo Cereals. Chapter 8, pp. 143-158. Woodhead Publishing. https://doi.org/https://doi.org/10.1016/B978-0-12-820089-6.00008-2.

[34] Rusli, L., Osman, N., Abdullah, R., Yaacob, J. S., Seow, A. (2021): Effects of Palm Kernel Biochar on The Physiological Responses and Root Profiles of Sendudok (*Melastoma malabathicum* L.) Grown on Acidic Soil. – Applied Ecology and Environmental Research 19(4): 2887-2903. https://doi.org/10.15666/aeer/1904_28872903.

[35] Shang, Y., Wan, Z., Zhao, C., Su, X., Liu, X. (2024): Effects of Water-Deficit Stress on the Growth and Physiological Characteristics of *Chloranthus spicatus* Seedlings. – Horticulturae 10(10): 1054. https://www.mdpi.com/2311-7524/10/10/1054.

[36] Shao, J., Bi, F., Li, M., Munir, R., Rasul, F., Tang, W., Liang, X., Aamer, M., Ahmad, N., Ullah, A., Alharbi, S. A., Alfarraj, S. (2024): Biochar improves morpho-physiological growth, osmolyte accumulation, nutrients balance, anti-oxidative defense and oil productivity of Brassica under flooding stress. – Notulae Botanicae Horti Agrobotanici Cluj-Napoca 52(3): 13835. https://doi.org/10.15835/nbha52313835.

[37] Stanhill, G. (1986): Water Use Efficiency. – In: Brady, N. C. (ed.) Advances in Agronomy 39: 53-85. Academic Press. https://doi.org/https://doi.org/10.1016/S0065-2113(08)60465-4.

[38] Sun, S., Hu, X., Wei, Y., Chen, X., Li, Y., Cao, J. (2024): Response of WUE of maize at ear stage to the coupling effect of CO_2 and temperature. – Heliyon 10(1): e23646. https://doi.org/https://doi.org/10.1016/j.heliyon.2023.e23646.

[39] Tardío, G., Mickovski, S. B. (2016): Implementation of eco-engineering design into existing slope stability design practices. – Ecological Engineering 92: 138-147. https://doi.org/https://doi.org/10.1016/j.ecoleng.2016.03.036.

[40] Uysal, E. (2018): Effects of Nitrogen Fertilization on the Chlorophyll Content of Apple [Azotlu Gübrelemenin Elmada Klorofil İçeriği Üzerine Etkileri]. – Meyve Bilimi 5(1): 12-17. https://dergipark.org.tr/tr/pub/meyve/issue/39881/400647.

[41] Vo, T. S., Ngo, D. H. (2019): The Health Beneficial Properties of *Rhodomyrtus tomentosa* as Potential Functional Food. – Biomolecules 9(2): 76. https://doi.org/10.3390/biom9020076.

[42] Wagner, W. L., Herbst, D. R., Sohmer, S. H. (1999): Rhodomyrtus. – In: Manual of the flowering plants of Hawaii, University of Hawaii Press, pp. 1356-1357.

[43] Wang, L., He, P., Hui, M., Li, H., Sun, A., Yin, H., Gao, X. (2024): Metabolomics combined with transcriptomics and physiology reveals the regulatory responses of soybean plants to drought stress. – Frontiers in Genetics 15. https://doi.org/10.3389/fgene.2024.1458656.

[44] Wei, M.-S., Chen, Z.-H., Ren, H., Yin, Z.-Y. (2009): Reproductive ecology of *Rhodomyrtus tomentosa* (Myrtaceae). – Nordic Journal of Botany 27(2): 154-160. https://doi.org/https://doi.org/10.1111/j.1756-1051.2009.00137.x.

[45] Winotai, A., Wright, T., Goolsby, J. (2009): Herbivores in Thailand on *Rhodomyrtus tomentosa* (Myrtaceae), an invasive weed in Florida. – Florida Entomologist 88: 104-105. https://doi.org/10.1653/0015-4040(2005)088[0104:HITORT]2.0.CO;2.

[46] Wu, Y., Wang, X., Zhang, L., Zheng, Y., Liu, X., Zhang, Y. (2023): The critical role of biochar to mitigate the adverse impacts of drought and salinity stress in plants. – Front Plant Sci 14: 1163451. https://doi.org/10.3389/fpls.2023.1163451.

[47] Yang, L., Ren, H., Liu, N., Wang, J. (2010): The shrub *Rhodomyrtus tomentosa* acts as a nurse plant for seedlings differing in shade tolerance in degraded land of South China. – Journal of Vegetation Science 21(2): 262-272. https://doi.org/https://doi.org/10.1111/j.1654-1103.2009.01140.x.

[48] Zhang, Y., Ding, J., Wang, H., Su, L., Zhao, C. (2020): Biochar addition alleviate the negative effects of drought and salinity stress on soybean productivity and water use efficiency. – BMC Plant Biol 20(1): 288. https://doi.org/10.1186/s12870-020-02493-2.

[49] Zoghi, Z., Hosseini, S. M., Kouchaksaraei, M. T., Kooch, Y., Guidi, L. (2019): The effect of biochar amendment on the growth, morphology and physiology of *Quercus castaneifolia* seedlings under water-deficit stress. – European Journal of Forest Research 138(6): 967-979. https://doi.org/10.1007/s10342-019-01217-y