Biochar-Improved Growth and Physiology of *Ehretia asperula* under Water-Deficit Condition

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**Abstract:** *Ehretia asperula*’s physiological responses to growth performance following oak-wood biochar application under water stress conditions (WSC) and no water stress conditions (non-WSC) were investigated in a pot experiment. Biochar (WB) was incorporated into the soil at concentrations of 0, 5, 10, 15, and 20 tons ha$^{-1}$ before transplanting *Ehretia asperula* in the pots. One month after transplanting, *Ehretia asperula* plants were put under water stress by withholding water for ten days. Water stress significantly decreased the growth and physiology of *Ehretia asperula*. Under WSC, the application of WB at the concentrations of 15 and 20 tons ha$^{-1}$ to the soil increased the plant height; number of leaves; fresh and dry weight of the roots, shoots, and leaves; $F_v/F_m$; chlorophyll content; leaf relative water content; and soil moisture as well as decreased the relative ion leakage. The application of WB enhanced drought tolerance in *Ehretia asperula* plants by lowering the wilting point. The findings suggest that WB application at the concentration of 15 tons ha$^{-1}$ could be recommended for ensuring the best physiological responses and highest growth of *Ehretia asperula* plants in water-deficit conditions.

**Keywords:** biochar; *Ehretia asperula*; growth; physiology; water deficit

1. Introduction

The genus *Ehretia* is mainly distributed in the tropical areas of Asia, Africa, and Northern America and exhibits valuable pharmacological properties [1,2]. *Ehretia asperula* Zoll. et Mor, a species in the genus *Ehretia*, was initially researched by Zollinger and Moritz [3]. In Vietnam, *Ehretia asperula* has been used in traditional medicine for the treatment of gastritis and pimples, as an antitumor medication, and in antioxidation by some ethnic minorities for a long time. Moreover, the product of alcoholic extraction from the bark of *Ehretia asperula* can be used in the treatment of liver cancer, nose cancer, colon cancer, and HIV H-9 resistance [4,5].

Drought is considered one of the major environmental factors responsible for the reduction in plant growth and yield [6,7]. Drought stress adversely affects soil properties, thus restraining plant metabolism and growth [6–9]. On the other hand, drought stress can alter the physiological characteristics of plant leaves, such as lowering the leaf photosynthetic and transpiration rate, and stomatal conductance and, consequently, restraining crop
productivity [10,11]. Therefore, it is important to develop techniques that can improve the water- and nutrient-holding capacities of soil, the yield of crops, and productivity.

Biochar is produced from a charred organic material in the presence of limited O$_2$ by a process called pyrolysis [12]. Recently, biochar has received considerable attention as a soil amendment [13]. The beneficial effects of biochar, such as improving soil physicochemical properties, including soil pH, cation exchange capacity, soil structure, water-holding capacity, and surface area under abiotic stresses, have been widely reported [14–17]. Furthermore, biochar enriches the physicochemical properties, biological properties, and soil enzymatic activity of the soil, increasing the water status of plants. Several studies have revealed that biochar improves soil quality and crop productivity under limited water conditions [18–21]. However, some results also reported that biochar does not always seem to be useful for crop growth [22,23]. We expected that the application of WB would alleviate the negative effects of water stress on plants and this beneficial effect of WB would increase as the WB rates increased. Therefore, the main objective of this study was conducted to investigate the efficacy of different biochar rates in improving the growth, physiology, and soil moisture of *Ehretia asperula* plant under water-deficit conditions.

2. Materials and Methods

2.1. Materials

The *Ehretia asperula* plants were propagated by stem cutting. The similar diameters of stem cuttings were inserted in a poly bag (70 mm in diameter × 100 mm in height) that had been filled with alluvial soil. Three months after rooting of stem cuttings, similar seedlings were transferred to the plastic pots (260 mm in diameter × 210 mm in height) which were filled with alluvial soil obtained from the experimental field at the Vietnam National University of Agriculture, Hanoi, Vietnam. Each pot contained 5 kg of dry alluvial soil. Biochar was incorporated into the plastic pots before transplanting the *Ehretia asperula* plants. The initial physical and chemical properties of the experimental soil were presented in Table 1.

### Table 1. The initial physical and chemical properties of the experimental soil.

| Parameters                  | Values |
|-----------------------------|--------|
| pH                          | 6.51   |
| Organic matter (%)          | 1.67   |
| Total N (%)                 | 0.09   |
| Total P (%)                 | 0.18   |
| Total K (%)                 | 1.34   |
| Exchangeable N (mg/100 g)   | 4.27   |
| Exchangeable P (mg/100 g)   | 50.02  |
| Exchangeable K (mg/100 g)   | 11.80  |

Oak-wood biochar (WB) was purchased from Gangwon Charmsoot Company, Hoengseong-gun, Gangwon Province, Korea. Biochar was produced at a temperature of 400 °C by pyrolysis. The proximate and ultimate analyses and physiochemical properties of oak-wood biochar were reported in Rajapaksha et al. [24] such as the pH: 10.17; EC: 2.15 dSm$^{-1}$, mobile matter: 31.42%; fixed matter: 56.04%; dissolved organic carbon (DOC): 14.6 mg L$^{-1}$; ash content: 5.03%; C: 88.71%; H: 1.21%; N: 0.36%; O: 9.72%; molar H/C: 0.16; Molar O/C: 0.08; specific surface area: 270.76 m$^{2}$ g$^{-1}$; pore volume: 0.12 cm$^{3}$ g$^{-1}$; pore diameter: 1.10 mm.

2.2. Experimental Procedure

The pot experiment was carried out in net house at the Vietnam National University of Agriculture, Hanoi, Vietnam. A split-plot experimental design was adopted with three replications (one replication with 8 plastic pots for each treatment). The amount of biochar used was the main factor in this experiment, and five application rates were
employed (0, 26.5, 53.1, 79.6, and 106.2 g biochar/pot, which corresponded to 0, 5, 10, 15, and 20 tons biochar ha$^{-1}$, respectively. Drought stress conditions, considered a subfactor, included water-stress condition (WSC) and no water-stress condition (non-WSC). A brief design of this experiment is summarized in Table 2. The non-WSC comprised tap-water use during the growth of *Ehretia asperula* plants. One plant in each pot was watered daily with 200 mL of tap water. The WSC comprised tap-water use for one month following transplanting and subsequent induction of WSC by withholding irrigation for 10 days (from 11 May to 20 May); after 10 days of water stress, the plants were re-watered.

Table 2. Experimental design of different treatments.

| Treating Conditions | Biochar Rates (tons ha$^{-1}$) |
|---------------------|-------------------------------|
| Non-WSC             | 0 5 10 15 20                  |
| WSC                 | 0 5 10 15 20                  |

One week after transplanting to pots, each plant was fertilized one time a week by replacing irrigation with 200 mL of a modified Hoagland nutrient solution with a composition of 0.3125 mM KNO$_3$, 0.45 mM Ca(NO$_3$)$_2$, 0.0625 mM KH$_2$PO$_4$, 0.125 mM MgSO$_4$·7H$_2$O, 11.92 µM H$_3$BO$_3$, 4.57 µM MnCl$_2$·4H$_2$O, 0.191 µM ZnSO$_4$·7H$_2$O, 0.08 µM CuSO$_4$·5H$_2$O, 0.024 µM (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O, 15.02 µM FeSO$_4$·7H$_2$O, and 23.04 µM Na$_2$EDTA·5H$_2$O.

2.3. Data Collection

**Growth Parameters**

The growth rates of plant height were recorded from 4 May to 30 May (four times in pre-treating water stress period, five times in water stress period, six times in after re-watering period).

The growth rates of plant height (cm/checking time) = \(PH_2 - PH_1\)

\(PH_2\): Plant height this checking time; \(PH_1\): Plant height last checking time

The plant height (cm), the number of leaves, and biomasses of *Ehretia asperula* plants were measured after 10 days of re-watering. The dry biomasses of roots, shoots, and leaves were determined, and their contents were measured by drying the fresh roots, shoots, and leaves samples at 80 °C until a steady weight in an oven (MOV-212F, Sanyo Electric Co., Ltd., Osaka, Japan).

**Physiological Attributes**

The methods used to determine the physiological attributes of the plants were from our previous work [25]. The quantum efficiency of photosystem II (F$_{v}$/F$_{m}$) and chlorophyll content were also recorded from 4 May to 30 May (four times in pre-treating water stress period, six times in water stress period, and five times in after re-watering period). Chlorophyll contents were measured by using a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Inc., Osaka, Japan).

The chlorophyll fluorescences (F$_{v}$/F$_{m}$) were measured by using a portable fluorometer (model OS-30p, Opti-Sciences Chlorophyll Fluorometer, Hudson, NY, USA). The initial fluorescence (F$_{0}$), maximum fluorescence (F$_{m}$), and potential quantum efficiency of photosystem II (F$_{v}$/F$_{m}$) were measured. From these fluorescence data, the following parameters were calculated: variable fluorescence (F$_{v}$ = F$_{m}$ – F$_{0}$) and the effective absorbed energy-conversion efficiency of photosystem II (F$_{v}$/F$_{0}$). Fluorescence determinations were performed between 08:00 h and 11:00 h on the same leaves. The leaves were submitted to a 30 min dark adaptation period using leaf-clip holders, so that all the reaction centers in the foliar region under analysis acquired the ‘open’ configuration, indicating the complete oxidation of the photosynthetic electron transport system.

The leaf relative water content was measured on the last day of withholding irrigation and 10 days after re-watering. To measure the leaf relative water content (RWC), 9 samples out of the 10 leaf discs, one per treatment, were obtained. Leaves were selected among the
youngest fully expanded leaves. The leaf discs were immediately weighed (fresh weight; FW). The samples were bathed in distilled water (temperature range of 25–30 °C) inside a porous platform in order to obtain the turgid weight (TW). At the end of the imbibition period, the leaf samples were placed in a pre-heated oven at 80 °C for 48 h to obtain the dry weight (DW). Values of FW, TW, and DW were used to calculate RWC using the equation below.

\[
RWC (%) = \frac{(FW - DW)}{(TW - DW)} \times 100
\]

The relative ion leakages in the leaves were measured on the last day of withholding irrigation. Relative ion leakage in the leaves was assessed by the leakage of electrolytes from the leaves of nine plants of similar size. Leakage of the electrolytes was determined with a conductivity meter (AG 8603, SevenEasy, Mettler Toledo, Switzerland). The leaf segments (disks of leaves with d = 1 cm²) were washed, blotted dry, weighted, and placed in stopped vials filled with an exact volume of deionized water. The vials were then incubated for 2 h in darkness with continuous shaking before the conduction (C1) was measured. The vials were heated to 80 °C for 2 h, and the conduction (C2) was measured again. The electrolyte leakage was expressed as a percentage of relative ion leakage, which was calculated according to the following equation [26]: Relative electrolyte leakage (%) = C1/C2 × 100.

The soil moistures were measured by an Aquaterr-Model T300 Moisture Measurement Instrument (Aquaterr Model T300, Aquaterr, Costa Mesa, CA, USA). Measurements were taken at 15 cm soil depth on the last day of withholding irrigation.

The percentage of wilted plants under drought stress was recorded from the fifth day to the tenth day of withholding water. The plant was only considered wilting when 75% of its leaves were withered.

2.4. Data Analysis and Statistics

Growth and physiological parameters (growth rate of plant height, plant height, number of leaves, Fv/Fm, chlorophyll content, and soil moisture) were gathered from 15 randomly selected plants per treatment to be used for the statistical analysis. The measurement of the percentage of the wilted plant was obtained from 24 plants to be used for the analysis. Nine plants per treatment were randomly selected for the statistical analysis of the remaining growth and physiology parameters (the dry biomasses of roots, shoots, and leaves; relative ion leakage; and leaf relative water content).

The data recorded for the growth and photosynthetic efficiency of *Ehretia asperula* were statistically analyzed coefficient of variation and least significant difference using IRISTAT 5.0. Mean separations were estimated using Duncan’s multiple range tests at \( p \leq 0.05 \).

3. Results

3.1. Effect of Biochar on Growth Characteristics

3.1.1. Effect of Biochar on Growth Rate of Plant Height

The growth performance of *Ehretia asperula* in different WB rates under WSC showed a reduction in the growth rate of plant height due to water stress compared with plants growing under non-WSC. The growth rate of the plant height of *Ehretia asperula* in all different WB rates under the non-WSC remained constant over time. In general, WSC reduced the growth rate of the plant height of *Ehretia asperula* while WB treatments increased the growth rate of plant height in both non-WSC and WSC. For instance, the highest value of growth rate of plant height was recorded with the 15 tons ha\(^{-1}\) WB treatment under both non-WSC and WSC (Figure 1).
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Figure 1. Cont.
3.1.2. Effect of Biochar on Plant Height and Number of Leaves

Water-stress conditions significantly reduced the plant height and number of leaves of *Ehretia asperula* plants in all without WB and WB treatments. However, WB treatments showed a significant increase in the plant height and number of leaves of *Ehretia asperula* plants in both non-WSC and WSC. The lowest values of the plant height and number of leaves of *Ehretia asperula* plants were recorded with the without-WB treatment under both non-WSC and WSC. The highest values of plant height of *Ehretia asperula* plants were recorded with the 15 tons ha⁻¹ WB treatment but were not statistically different with plants in the 20 tons ha⁻¹ WB treatment under both non-WSC and WSC. The highest values of the number of leaves of *Ehretia asperula* plants were recorded with the 15 tons ha⁻¹ WB treatment but not statistically different with plants in the 20 tons ha⁻¹ WB treatment under WSC (Table 3).

Table 3. Effect of biochar on plant height and number of leaves of *Ehretia asperula* under non-WSC and WSC.

| Biochar Rates (tons ha⁻¹) | Treating Conditions | Plant Height (cm) | Leaf Number (Leaves) |
|--------------------------|---------------------|-------------------|----------------------|
|                          | Non-WSC             | 36.10 bc          | 24.20 c              |
|                          | WSC                 | 27.26 e           | 19.40 d              |
| 5                        | Non-WSC             | 39.08 b           | 26.40 c              |
|                          | WSC                 | 31.46 d           | 24.00 c              |
| 10                       | Non-WSC             | 45.36 a           | 32.40 b              |
|                          | WSC                 | 32.68 cd          | 24.60 c              |
| 15                       | Non-WSC             | 47.50 a           | 41.20 a              |
|                          | WSC                 | 38.96 b           | 26.80 c              |
| 20                       | Non-WSC             | 44.90 a           | 35.80 b              |
|                          | WSC                 | 37.45 b           | 25.00 c              |

**Figure 1.** Growth rate of the plant height of *Ehretia asperula* in response to biochar treatments in concentrations of 0 ton ha⁻¹ (A), 5 tons ha⁻¹ (B), 10 tons ha⁻¹ (C), 15 tons ha⁻¹ (D), and 20 tons ha⁻¹ (E) under the non-WSC and WSC. Vertical bars represent mean ± SD, n = 15.
Table 3. Cont.

| Biochar Rates (tons ha\(^{-1}\)) | Treating Conditions | Plant Height (cm) | Leaf Number (Leaves) |
|----------------------------------|---------------------|------------------|----------------------|
| CV%                              | 0                   | 31.68 D          | 21.80 D              |
| LSD\(_B\times T\) 0.05            | 5                   | 35.27 C          | 25.20 CD             |
| Average of biochar rates         | 10                  | 39.02 B          | 28.50 BC             |
|                                  | 15                  | 43.23 A          | 34.00 A              |
|                                  | 20                  | 41.18 AB         | 30.40 AB             |
| LSD\(_B\) 0.05                   |                     | 2.59             | 3.05                 |
| Average of conditions            | Non-WSC             | 42.59 A          | 32.00 A              |
|                                  | WSC                 | 33.56 B          | 23.96 B              |
| LSD\(_T\) 0.05                   |                     | 1.64             | 1.93                 |

CV, coefficient of variation; LSD, least significant difference; T, treating conditions (non-WSC and WSC); B, biochar rates. Different lowercase letters show interaction significance among biochar rates and treating conditions at \(p < 0.05\). Different capital letters show significance among biochar rates or significance between treating conditions by Duncan’s multiple range tests at \(p \leq 0.05\).

3.1.3. Effect of Biochar on Dry Weight of Roots, Shoots and Leaves

Biochar application significantly increased the dry biomasses of roots, shoots, and leaves of *Ehretia asperula* plants under both non-WSC and WSC. However, WSC significantly decreased the dry biomasses of roots, shoots, and leaves of *Ehretia asperula* plants in all without WB and WB treatments. The lowest values of the dry weight of roots, shoots, and leaves of *Ehretia asperula* plants were recorded with the without-WB treatment under both non-WSC and WSC. The highest values of dry biomasses of roots of *Ehretia asperula* plants were observed in the 15 tons ha\(^{-1}\) WB treatment but not statistically different with plants in the 20 tons ha\(^{-1}\) WB treatment under WSC. The highest values of dry biomasses of shoots and leaves of *Ehretia asperula* plants were observed in the 15 tons ha\(^{-1}\) WB treatment but not statically different with plants in the 20 tons ha\(^{-1}\) WB treatment under non-WSC and WSC (Table 4).

Table 4. Effect of biochar on dry weight of *Ehretia asperula* under non-WSC and WSC.

| Biochar Rate (tons ha\(^{-1}\)) | Treating Conditions | Root (g Plant\(^{-1}\)) | Shoot (g Plant\(^{-1}\)) | Leaves (g Plant\(^{-1}\)) |
|---------------------------------|---------------------|------------------------|-------------------------|-------------------------|
| 0                               | Non-WSC             | 0.27 d                 | 2.61 d                  | 1.33 e                  |
| 5                               | WSC                 | 0.17 f                 | 1.61 f                  | 1.18 f                  |
| 10                              | Non-WSC             | 0.31 c                 | 3.14 c                  | 1.43 d                  |
| 15                              | WSC                 | 0.19 ef                | 2.32 e                  | 1.22 f                  |
| 20                              | Non-WSC             | 0.32 c                 | 3.43 b                  | 1.53 c                  |
|                                 | WSC                 | 0.21 e                 | 2.77 d                  | 1.48 cd                 |
| CV%                             |                      | 5.7                    | 4.8                     | 2.8                     |
| LSD\(_B\times T\) 0.05          | 0.026               | 0.248                  | 0.072                   |
| Average of biochar rates        | 0                   | 0.22 D                 | 2.11 D                  | 1.26 C                  |
|                                 | 5                   | 0.25 C                 | 2.73 C                  | 1.32 C                  |
|                                 | 10                  | 0.26 C                 | 3.10 B                  | 1.50 B                  |
|                                 | 15                  | 0.32 B                 | 3.73 A                  | 1.79 A                  |
|                                 | 20                  | 0.30 A                 | 3.67 A                  | 1.77 A                  |
3.2. Effect of Biochar on Physiology Characteristics

3.2.1. Effect of Biochar on Chlorophyll Content

To examine the physiological performance of *Ehretia asperula* plants under WSC, we measured the change in chlorophyll content of *Ehretia asperula* plants in different WB rates between before (4 May) and after (30 May) water-stress conditions were applied. The SPAD values revealed that the chlorophyll content ranged from 48.76 to 56.94 and from 51.18 to 57.02 at the beginning of the pre-treatment period (from 4 May to 10 May) for both groups under non-WSC and WSC, respectively, indicating that there were no significant differences among growing plants under the experimental conditions. The SPAD values sharply decreased to 36.40–41.56 in the leaves of *Ehretia asperula* plants growing under WSC (20 May). The lowest SPAD values of *Ehretia asperula* plants were recorded with the without-WB treatment under both non-WSC and WSC. On average, the treatments with WB increased the chlorophyll content in the leaves of *Ehretia asperula* plants (Figure 2).

![Figure 2](image-url)

**Figure 2.** Effect of biochar on SPAD values of *Ehretia asperula* under non-WSC (A) and WSC (B). Vertical bars represent the mean ± SD, *n* = 15.

Table 4. Cont.

| Biochar Rate (tons ha⁻¹) | Treating Conditions | Root (g Plant⁻¹) | Shoot (g Plant⁻¹) | Leaves (g Plant⁻¹) |
|--------------------------|---------------------|------------------|------------------|-------------------|
|                          | LSDₜ₀.₀₅            | 0.018            | 0.175            | 0.512             |
| Average of conditions    | Non-WSC             | 0.32 A           | 3.40 A           | 1.60 A            |
|                          | WSC                 | 0.22 B           | 2.74 B           | 1.46 B            |
|                          | LSDₜ₀.₀₅            | 0.011            | 0.111            | 0.032             |

CV, coefficient of variation; LSD, least significant difference; T, treating conditions (non-WSC and WSC); B, biochar rates. Different lowercase letters show interaction significance among biochar rates and treating conditions at *p* < 0.05. Different capital letters show significance among biochar rates or significance between treating conditions by Duncan’s multiple range tests at *p* ≤ 0.05.
3.2.2. Effect of Biochar on Photosynthetic Efficiency ($F_v/F_m$)

To examine the effect of water stress on physiological performance, we measured the changes in the quantum efficiency of photosystem II ($F_v/F_m$) of *Ehretia asperula* plants in different WB rates from before (4 May) to after (30 May) the WSC was applied. The values of $F_v/F_m$ ranged from 0.66 to 0.77 and from 0.67 to 0.75 at the beginning of the pre-treatment period (from 4 May to 10 May) for both groups under non-WSC and WSC, respectively. It was found that the fluctuations in the values of $F_v/F_m$ were observed in all different WB rates under both the non-WSC and WSC; however, $F_v/F_m$ showed a sharp decrease in plants that had under WSC. Biochar treatments improved the $F_v/F_m$ of *Ehretia asperula* grown under both the non-WSC and WSC. The lowest values of $F_v/F_m$ of *Ehretia asperula* plants were recorded with the without-WB treatment under both non-WSC and WSC. The high values of $F_v/F_m$ of *Ehretia asperula* plants were recorded with the 15 and 20 tons ha$^{-1}$ WB treatments under both non-WSC and WSC (Figure 3).

Figure 3. Effect of biochar on $F_v/F_m$ of *Ehretia asperula* under the non-WSC (A) and WSC (B). Vertical bars represent the mean ± SD, $n = 15$.

3.2.3. Effect of Biochar on Leaf Relative Water Content

WSC decreased the leaf relative water content of *Ehretia asperula*, whereas WB treatments increased the leaf relative water content significantly. The highest values of the leaf relative water content were recorded for the 15 tons ha$^{-1}$ WB treatment under both non-WSC and WSC after 10 days withholding irrigation and after 10 days re-watering. The lowest values of the leaf relative water content were observed in plants growing in without WB treatment (0 tons ha$^{-1}$ of WB treatment), followed by those grown with the 5 tons ha$^{-1}$ WB treatment under the non-WSC and WSC. Although biochar noticeably increased the leaf relative water content, the difference in the leaf relative water content among the 15 and 20 tons ha$^{-1}$ WB treatments was not significant under both the non-WSC and WSC after 10 days withholding irrigation and after 10 days re-watering (Figure 4).
3.2.4. Effect of Biochar on Relative Ion Leakage

WSC increased the relative ion leakage of *Ehretia asperula* leaves in different WB rates (Figure 5). The highest values of relative ion leakage were found to be in plants without WB treatment under the non-WSC and WSC. The application of WB significantly decreased the relative ion leakage under non-WSC and WSC. The 15 tons ha\(^{-1}\) WB treatment had the lowest relative ion leakage values after 10 days withholding irrigation, although no significant difference in the reduction in the relative ion leakage in the plants grown with the 15 and 20 tons ha\(^{-1}\) WB treatments after 10 days withholding irrigation under both non-WSC and WSC was observed (Figure 5).

Figure 4. Effect of biochar on leaf relative water content of *Ehretia asperula* after 10 days without irrigation (A) and 10 days after re-watering (B). Vertical bars represent the mean ± SD, \(n = 9\).

Figure 5. Effect of biochar on relative ion leakage of *Ehretia asperula* after 10 days without irrigation. Vertical bars represent the mean ± SD, \(n = 9\).
3.3. Effect of Biochar on Soil Moisture

The application of WB significantly increased the soil moisture under WSC. The soil moisture increased with increased WB rates under WSC. The soil enriched with the 20 tons ha\(^{-1}\) WB treatment gave the highest soil moisture value after 10 days of withholding irrigation. However, no significant difference in moisture of the soil enriched with the 15 and 20 tons ha\(^{-1}\) WB treatments after 10 days withholding irrigation in WSC was observed. The lowest values of the soil moisture were recorded with the without-WB treatment under WSC (Figure 6).

![Figure 6. Effect of biochar on soil moisture of *Ehretia asperula* after 10 days without irrigation. Vertical bars represent the mean ± SD, \(n = 9\).](image)

3.4. Effect of Biochar on Wilted Plant

The addition of Biochar to the soil enhanced the drought tolerance of *Ehretia asperula* plants by lowering the wilting point. In the without biochar treatment, the wilting point was reached on the fifth day withholding irrigation, but in the 15 and 20 tons ha\(^{-1}\) WB treatment groups, the wilting point was reached on the sixth day withholding irrigation. On the other hand, 100% of the plants had wilted by the eighth day withholding irrigation in the without biochar treatment, but in the 15 and 20 tons ha\(^{-1}\) WB treatment groups, only 80% of the plants had wilted by the tenth day withholding irrigation (Table 5).

| Biochar Rates (tons ha\(^{-1}\)) | Percentage of Wilted Plant after without Irrigation (%) | Fifth Day | Sixth Day | Seventh Day | Eighth Day | Ninth Day | Tenth Day |
|-------------------------------|-------------------------------------------------------|-----------|-----------|-------------|------------|-----------|-----------|
| 0                             |                                                       | 54.17     | 66.67     | 75.00       | 100.00     | -         | -         |
| 5                             |                                                       | 41.67     | 54.17     | 66.67       | 100.00     | -         | -         |
| 10                            |                                                       | 20.83     | 33.33     | 58.33       | 75.00      | 91.67     | 100.00    |
| 15                            |                                                       | 0.00      | 20.83     | 33.33       | 41.67      | 66.67     | 79.17     |
| 20                            |                                                       | 0.00      | 20.83     | 33.33       | 41.67      | 58.33     | 79.17     |

Table 5. Effect of biochar on percentage of wilted plants of *Ehretia asperula* under WSC.
4. Discussion

Drought stress affects the growth, phenology, water and nutrient relations, photosynthesis, assimilate-partitioning, and respiration in plants, which results in the production of smaller organs [27]. These phenomena are controlled by physiological factors, such as plant hydraulic status, phytohormones, osmotic adjustment, and reactive oxygen species signaling [28,29]. In our study, water stress significantly decreased the growth and photosynthetic efficiency of *Ehretia asperula*, as demonstrated by the analysis of parameters such as plant height, number of leaves, dry biomasses of root, shoot, and leaves; $F_{v}/F_{m}$, chlorophyll content, and leaf relative water content.

Biochar not only improves the soil structure, fertility, and ion transfer ability but also increased the activity of microbes and nutrient holding and exchange capacity of the soil. Therefore, biochar addition is proposed as an effective management to improve crop performance [21], as supported by the results we obtained from the experiments on *Ehretia asperula* plants, for which all biochar treatments clearly improved the growth characteristics such as plant height; number of leaves; and dry biomasses of root, shoot and leaves. These results agree with previous data obtained by several researchers who reported that the application of biochar increased the plant height and leaf area of okra [30] and maize [31] under drought-stress conditions. In addition, Olmo et al. [32] reported that the application of biochar increased the biomass of field-grown wheat under semiarid Mediterranean conditions. Moreover, Vaccari et al. [33] also found that the application of biochar in silt clay soil increased the growth of tomato plants when compared with the control.

On the other hand, several studies have reported that biochar addition improved photosynthesis in plants under drought-stress conditions [18,34,35]. Biochar increased photosynthesis and water-use efficiency of okra under drought stress when compared with the control [30]. In addition, biochar increased the water-use efficiency of *Chenopodium quinoa* wild under drought stress [36] and increased the water-use efficiency of maize in sandy soil [37]. Therefore, in our study, WB treatments inversely increased the SPAD values, photosynthetic efficiency ($F_{v}/F_{m}$), and leaf relative water content of *Ehretia asperula* significantly. These results agree with previous results obtained by Haider et al. [31], who reported that biochar application increased the leaf relative water content, transpiration rate, and osmotic potential of drought-stressed maize when compared with the control. In addition, Akhtar et al. [38] also showed that the application of biochar significantly improved physiological characteristics such as chlorophyll content, stomatal conductance, photosynthetic rate, water-use efficiency, and leaf relative water content as well as increased the stomatal density of tomato leaves under drought stress conditions.

Biochar improves the physical and biological properties of soils and increases the water-holding capacity of soil under drought stress conditions [39]. In our study, the application of WB significantly increased soil moisture under WSC. On the other hand, WB application enhanced the drought tolerance of *Ehretia asperula* plants by lowering the wilting point. These results agree with previous results obtained by Akhtar et al. [38] and Chintala et al. [40], who found that biochar application greatly increased the water-holding capacity of soil and, consequently, enhanced plant physiological characteristics such as chlorophyll content, stomatal conductance, photosynthetic rate, and leaf relative water content. In addition, Mulcahy et al. [41] also demonstrated that biochar application in sandy soil significantly increased tomato seedling resistance to wilting.

5. Conclusions

Application of WB to the soil significantly increased the growth and physiology of *Ehretia asperula* plants under both non-WSC and WSC. On the other hand, WB addition enhanced the soil moisture and drought tolerance of *Ehretia asperula* plants under WSC by lowering the wilting point. The current study suggests that applying WB at a rate of 15 tons ha$^{-1}$ could be recommended for ensuring the best growth and physiological responses of *Ehretia asperula* plants under WSC.
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References

1. Li, L.; Peng, Y.; Yao, X.; Xu, L.J.; Wulan, T.; Liu, Y.; Shi, R.B.; Xiao, P.G. Chemical constituents and biological activities of plants from the genus Ehretia Linn. Chin. Herb. Med. 2010, 2, 106–111. [CrossRef]
2. Shukla, A.; Kaur, A. A Systematic Review of Traditional Uses Bioactive Phytoconstituents of Genus Ehretia. Asian J. Pharm. Clin. Res. 2018, 11, 88–100. [CrossRef]
3. Gottschling, M.; Luebert, F.; Hilger, H.H.; Miller, J.S. Molecular delimitations in the Ehretiaceae (Boraginales). Mol. Phylogenet. Evol. 2014, 72, 1–6. [CrossRef] [PubMed]
4. Chien, Y.-C.; Lin, C.-H.; Chiang, M.Y.; Chang, H.-S.; Liao, C.-H.; Chen, I.-S.; Peng, C.-F.; Tsai, I.-L. Secondary metabolites from the root of Ehretia longiflora and their biological activities. Phytochemistry 2012, 80, 50–57. [CrossRef] [PubMed]
5. Ferreres, F.; Vinholes, J.; Gil-Izquierdo, A.; Valentrà, P.; Gonçalves, R.F.; Andrade, P.B. In vitro studies of α-glucosidase inhibitors and antiradical constituents of Glandora diffusa (Lag.) D.C. Thomas infusion. Food Chem. 2013, 136, 1390–1398. [CrossRef]
6. Bodner, G.; Nakhfoorosh, A.; Kaul, H.-P. Management of crop water under drought: A review. Agron. Sustain. Dev. 2015, 35, 401–442. [CrossRef]
7. Wang, L.; Sun, X.; Li, S.; Zhang, T.; Zhang, W.; Zhai, P. Application of Organic Amendments to a Coastal Saline Soil in North China: Effects on Soil Physical and Chemical Properties and Tree Growth. PLoS ONE 2014, 9, e89185. [CrossRef]
8. Ghanbary, E.; Kouchaksaraei, M.T.; Guidi, L.; Mirabolfathy, M.; Etemad, V.; Sanavi, S.A.M.M.; Struve, D. Change in biochemical parameters of Persian oak (Quercus brantii Lindl.) seedlings inoculated by pathogens of charcoal disease under water deficit conditions. Trees 2018, 32, 1595–1608. [CrossRef]
9. Jafarnia, S.; Akbarinia, M.; Hosseinpour, B.; Sanavi, S.M.; Salami, S. Effect of drought stress on some growth, morphological, physiological, and biochemical parameters of two different populations of Quercus brantii. IForest Biogeosci. For. 2018, 11, 212–220. [CrossRef]
10. Mathobo, R.; Marais, D.; Steyn, M. The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (Phaseolus vulgaris L.). Agric. Water Manag. 2017, 180, 118–125. [CrossRef]
11. Hussain, M.; Farooq, S.; Hasan, W.; Ul-Allah, S.; Tanveer, M.; Farooq, M.; Nawaz, A. Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. Agric. Water Manag. 2018, 201, 152–166. [CrossRef]
12. Abel, S.; Peters, A.; Trinks, S.; Schonsky, H.; Facklam, M.; Wessolek, G. Impact of biochar and hydrochar addition on water retention and water repellency of sandy soil. Geoderma 2013, 202–203, 183–191. [CrossRef]
13. Saifullah; Dahlawi, S.; Naeem, A.; Rengel, Z.; Naidu, R. Biochar application for the remediation of salt-affected soils: Challenges and opportunities. Sci. Total Environ. 2018, 625, 320–335. [CrossRef] [PubMed]
14. Hammer, E.C.; Forstreuter, M.; Rillig, M.; Kohler, J. Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress. Appl. Soil Ecol. 2015, 96, 114–121. [CrossRef]
15. Andreveni, M.; Maienza, A.; Genesio, L.; Miglietta, F.; Pellegrini, S.; Vaccari, E.; Vignozzi, N. Field application of pelletized biochar: Short term effect on the hydrological properties of a silty clay loam soil. Agric. Water Manag. 2016, 163, 190–196. [CrossRef]
16. Bamminger, C.; Poll, C.; Sixt, C.; Högy, P.; Wüst, D.; Kandeler, E.; Marhan, S. Short-term response of soil microorganisms to biochar addition in a temperate agroecosystem under soil warming. Agric. Ecosyst. Environ. 2016, 233, 308–317. [CrossRef]
17. Lim, T.; Spokas, K.; Feyereisen, G.; Novak, J. Predicting the impact of biochar additions on soil hydraulic properties. Chemosphere 2016, 142, 136–144. [CrossRef] [PubMed]
18. Paneque, M.; De la Rosa, J.M.; Franco-Navarro, J.D.; Colmenero-Flores, J.M.; Knicker, H. Effect of biochar amendment on morphology, productivity and water relations of sunflower plants under non-irrigation conditions. Catena 2016, 147, 280–287. [CrossRef]
19. Zoghi, Z.; Hosseini, S.M.; Kouchakzareei, M.T.; Koоч, Y.; Guidi, L. The effect of biochar amendment on the growth, morphology and physiology of Quercus castaneifolia seedlings under water-deficit stress. *Eur. J. For. Res.* 2019, 138, 967–979. [CrossRef]

20. Haider, I.; Raza, M.A.S.; Iqbal, R.; Aslam, M.U.; Habib-Ur-Rahman, M.; Raja, S.; Khan, M.T.; Waqas, M.; Ahmad, S. Potential effects of biochar application on mitigating the drought stress implications on wheat (*Triticum aestivum* L.) under various growth stages. *J. Sanit. Chem. Soc.* 2020, 24, 974–981. [CrossRef]

21. Zhang, Y.; Ding, J.; Wang, H.; Su, L.; Zhao, C. Biochar addition alleviate the negative effects of drought and salinity stress on soybean productivity and water use efficiency. *BMC Plant Biol.* 2020, 20, 1–11. [CrossRef] [PubMed]

22. Libutti, A.; Trott, V.; Rivelli, A.R. Biochar, Vermicompost, and Compost as Soil Organic Amendments: Influence on Growth Parameters, Nitrate and Chlorophyll Content of Swiss Chard (*Beta vulgaris* L. var. *cylina*). *Agronomy* 2020, 10, 346. [CrossRef]

23. Libutti, A.; Rivelli, A. Quanti-Qualitative Response of Swiss Chard (*Beta vulgaris* L. var. *cylina*) to Soil Amendment with Biochar-Compost Mixtures. *Agronomy* 2021, 11, 307. [CrossRef]

24. Rajapaksha, A.U.; Ok, Y.S.; El-Naggar, A.; Kim, H.; Song, F.; Kang, S.; Tsang, Y.F. Dissolved organic matter characterization of biochars produced from different feedstock materials. *J. Environ. Manag.* 2019, 233, 393–399. [CrossRef]

25. Vu, N.-T.; Park, J.-M.; Jang, D.-C.; Tran, A.-T.; Kim, I.-S. Effect of Abscisic Acid on Growth and Physiology of Arabica Coffee Seedlings under Water Deficit Condition. *Sains Malays.* 2020, 49, 1499–1508. [CrossRef]

26. Zhao, M.; Zhao, X.; Wu, Y.; Zhang, L. Enhanced sensitivity to oxidative stress in an Arabidopsis nitric oxide synthase mutant. *J. Plant Physiol.* 2007, 164, 735–745. [CrossRef]

27. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Plant drought stress: Effects, mechanisms and management. *Agron. Sustain. Dev.* 2009, 29, 185–212. [CrossRef]

28. Tardieu, F.; Parent, B.; Caldeira, C.F.; Welcker, C.; Oh, D.-H.; Hong, H.; Lee, S.Y.; Yun, D.-J.; Bohnert, H.J.; Dassanayake, M. Genetic and Physiological Controls of Growth under Water Deficit. *Plant Physiol.* 2014, 164, 1628–1635. [CrossRef]

29. Khan, M.I.R.; Asgher, M.; Fatma, M.; Per, T.S.; A Khan, N. Drought stress vis a vis plant functions in the era of climate change. *Clim. Chang. Environ. Sustain.* 2015, 3, 13. [CrossRef]

30. Batool, A.; Taj, S.; Rashid, A.; Khalid, A.; Qadeer, S.; Ghufran, M.A. Potential of soil amendments (Biochar and Gypsum) in increasing water use efficiency of Abelmoschus esculentus L. Moench. *Front. Plant Sci.* 2015, 6, 733. [CrossRef]

31. Haider, G.; Koyro, H.-W.; Azam, F.; Steffens, D.; Müller, C.; Kammann, C. Biochar but not humic acid product amendment affected maize yields via improving plant-soil moisture relations. *Plant Soil* 2015, 395, 141–157. [CrossRef]

32. Olmo, M.; Alburquerque, J.A.; Barrón, V.; del Campillo, M.C.; Gallardo, A.; Fuentes, M.; Villar, R. Wheat growth and yield responses to biochar addition under Mediterranean climate conditions. *Biol. Fertil. Soils* 2014, 50, 1177–1187. [CrossRef]

33. Vacca, F.P.; Maienza, A.; Miglietta, F.; Baronti, S.; Di Lonardo, S.; Giagnoni, L.; Lagomarsino, A.; Pozzi, A.; Pusceddu, E.; Ranieri, R.; et al. Biochar stimulates plant growth but not fruit yield of processing tomato in a fertile soil. *Agric. Ecosyst. Environ.* 2015, 207, 163–170. [CrossRef]

34. Lyu, S.; Du, G.; Liu, Z.; Zhao, L.; Lyu, D. Effects of biochar on photosystem function and activities of protective enzymes in Pyrus ussuriensis Maxim. under drought stress. *Acta Physiol. Plant.* 2016, 38, 220. [CrossRef]

35. Xiao, Q.; Zhu, L.-X.; Shen, Y.-F.; Li, S.-Q. Sensitivity of soil water retention and availability to biochar addition in rainfed semi-arid farmland during a three-year field experiment. *Field Crop. Res.* 2016, 196, 284–293. [CrossRef]

36. Kammann, C.I.; Linsel, S.; Gößling, J.W.; Koyro, H.-W. Influence of biochar on drought tolerance of Chenopodium quinoa Willd and on soil–plant relations. *Plant Soil* 2011, 345, 195–210. [CrossRef]

37. Uzoma, K.C.; Inoue, M.; Andry, H.; Fujimaki, H.; Zahoor, A.; Nishihara, E. Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use Manag.* 2011, 27, 205–212. [CrossRef]

38. Ali, S.; Rizwan, M.; Qayyum, M.F.; Ok, Y.S.; Ibrahim, M.; Riaz, M.; Arif, M.S.; Hafeez, F.; Al-Wabel, M.I.; Shahzad, A.N. Biochar soil amendment on alleviation of drought and salt stress in plants: A critical review. *Environ. Sci. Pollut. Res.* 2017, 24, 12700–12712. [CrossRef]

39. Chintala, R.; Molineno, J.; Schumacher, T.E.; Malo, D.D.; Julson, J.L. Effect of biochar on chemical properties of acidic soil. *Arch. Agron. Soil Sci.* 2014, 60, 393–404. [CrossRef]

40. Mulcahy, D.N.; Mulcahy, D.L.; Dietz, D. Biochar soil amendment increases tomato seedling resistance to drought in sandy soils. *J. Arid. Environ.* 2013, 88, 222–225. [CrossRef]