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The effects of biochar grain size on radish plants under low water availability

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Abstract: Low water availability is a significant constraint on global crop production. Exploration is needed regarding plant responses to drought in interaction with biochar, encompassing optimised water use and carbon allocation strategies. The size of the biochar particles also plays an important role, especially in influencing the dynamics of water and plant growth. This study explored the potential impact of biochar treatment on radish growth and drought tolerance. Finer biochar particles lead to the most substantial available water content for plants, increasing at around 30%, while medium and larger fractions increase by about 22% and 16%, respectively, compared to control soil. The chlorophyll fluorescence technique showed improved water management of drought stress at larger fractions of biochar. Our research underscores the potential of biochar treatments for environmental stresses and water scarcity in modern agriculture.

Keywords: biochar fraction size; water stress; carbon rich material; silt loam soil

Drought is a significant constraint on global crop production, with predictions from crop growth models indicating its severity will worsen in the future (Liu et al. 2017). To safeguard biomass production and conserve water resources, exploring plant adaptations that can mitigate the adverse effects of drought is imperative. An effective approach to achieve this is the application of biochar. Biochar, a solid carbonaceous material with remarkable carbon stability, is derived through biomass pyrolysis in a controlled oxygen-deficient environment at temperatures ranging from 300 to 1 000 °C (Verheijen et al. 2010). The analysis of plant responses to drought after adding biochar is necessary to comprehend the physiological basis of improved crop yield and stability. Over time, plants have evolved sophisticated strategies for adjustment and survival in their environment. These strategies include optimising water availability for root establishment, minimising transpiration to prevent dehydration, adjusting photosynthesis to provide metabolic substrates, and increasing carbon allocation to growing tissues and storage organs (Condon et al. 2004). Studies have consistently shown that biochar has a significant impact on enhancing plant growth, as it effectively modifies the physio-chemical properties of the substrates (Zhang et al. 2021, Krzyszczak et al. 2022). Furthermore, numerous reports have highlighted the positive outcomes resulting from biochar

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amendment in substrates at drought stress (Obadi et al. 2023, Safari et al. 2023). The soil water retention property plays a significant role in soil management in defining available water content (AWC) in the soil for plants. Lately, increasing attention has been paid to the improvement of water retention and notable improvements in AWC after biochar amendment were reported in the case of different soil types (Vitková et al. 2017, Seyedsadr et al. 2022). Soil water content characteristics, which indicate the status of AWC, are known as the soil water constants (hydrolimits). AWC can be estimated from the soil water retention curve as the difference between constants the field capacity (θ_{FC}) and the wilting point (θ_{WP}) (Novák and Hlaváčiková 2019). Biochar particle size plays an important role in changes in various soil properties. Nevertheless, there is little research on the probable influences of the grain size of biochar on soil (Liu et al. 2017, Razzaghi et al. 2020). If they exist, they focus on soil water properties and not on the impact on the growth of plants. The effect of biochar texture size on water dynamics was investigated by Conte and Nestle (2015). Their results proved that 3D exchange between bound and bulk water predominantly occurred in the coarsest fraction. However, as porosity decreased, water motion was mainly associated to a restricted 2D diffusion among the surface-site pores and the bulk-site ones. The pot experiment of Glab et al. (2016) indicated that biochar application significantly improved the physical properties of the tested sandy soil. The basic soil physical parameters, such as bulk density and total porosity, were not only dependent on the rate but also on the fraction size of the biochar. Biochar application increased the AWC, especially when the finest fraction was used.

Radish (*Raphanus sativus* L.) is a quick-growing crop commonly used in scientific research. It is a great choice for small-scale producers, as it can be grown between longer cycle crops, resulting in a quick payback of around 30 days. Additionally, it has low drought tolerance (Sousa and Figueiredo 2016).

The specific objectives of this study were to (*i*) investigate the effects of adding different grain sizes of biochar particles to the potting substrate and (*ii*) determine the individual effect of biochar-amended potting substrate on the growth of radish.

MATERIAL AND METHODS

Pot experiment. Soil for the experiment was obtained from the experimental site located in Dolná Malanta (SVK) (48°19'00"N, 18°09'00"E). This area belongs to the Slovak University of Agriculture (SUA) in Nitra and is used for conventional agricultural production. Toková et al. (2020) classified soil as Haplic Luvisol according to the Soil Taxonomy with the initial soil organic carbon content of 9.13 g/kg, pH of 5.71 (weakly acidic). Šimanský and Klimaj (2017) classified this soil as silty loam soil based on USDA classification (the content of sand 15.2%, silt 59.9% and clay 24.9%).

The disturbed soil sample was air-dried and passed through a 2-mm sieve. The fractionation of the produced biochar to the different particle sizes was carried out by dry sieving using a set of 125 μm and 2 mm sieves. The resulting biochar had the following size fractions: < 125 μm, 125 μm–2 mm and > 2 mm, referred to hereafter as B1, B2 and B3, respectively. Then, soil was mixed with each biochar size at a concentration of 1.5% (weight of biochar/total weight). Four treatments were prepared: control (pure soil with zero application of biochar), soil + biochar < 125 μm (S + B1), soil + biochar 125 μm – 2 mm (S + B2) and soil + biochar > 2 mm (S + B3). Each treatment was replicated five times. The soil pots were placed in controlled laboratory conditions.

Biochar characteristics. The used biochar was produced from willow, cv. Tordis (*Salix Schwerinii* × *Salix viminalis*). It was made in the UNYPIR reactor at a pyrolysis temperature of 300 °C and 101 kPa pressure for 8–10 min. The reactor is part of the AgroBioTech centre and belongs to SUA (Nitra, Slovak Republic). This biochar is a non-commercial product produced on a small scale because of research. Table 1 shows the basic properties of biochar.

Retention characteristics. The retention characteristics of soil and soil + biochar mixtures were determined based on soil water retention curves. They were measured in the pressure plate apparatus (STN EN ISO 11274: 2014) made by Soil Moisture Equipment Corp. (Santa Barbara, USA). All measured samples were fully saturated and moved to the pressure plate apparatus, and a total of nine measurement points were used at pressure potentials of 0, 6, 10, 33, 56, 100, 300, 480 and 1 500 kPa. Based

Table 1. Basic chemical analyses of used biochar

pH	Ash	C	H	N
	(%)			
9.14	6.16	82.2	2.74	0.86

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on measured data, the water retention curve values were fitted using the unimodal van Genuchten model (Van Genuchten 1980). Available water content for plants was calculated based on Eq. (1):

$$\theta_{AWC} = \theta_{FC} - \theta_{WP} \quad (1)$$

where: θ_{AWC} – available soil water content for plants (m^3/m^3); θ_{FC} – soil water content by field capacity constant (m^3/m^3); θ_{WP} – soil water content by wilting point constant (m^3/m^3). The θ_{FC} and θ_{WP} values were determined from soil water retention curves at –33 kPa (pF 2.5) and –1 500 kPa (pF 4.18), respectively.

Plant growth experiment. The study was conducted in a pot laboratory experiment. In the experiment was used radish seeds (*Raphanus sativus* L. var. *sativus*) cultivar Lada from commercial supplier Moravoseed CZ a.s. The used cultivar was round, bright red with a white core. Five plants and the control were planted in 9 × 7 cm plastic pots for each variant. The plants were grown under natural photoperiodic conditions. Laboratory conditions were set at 23 °C air temperature and with air relative humidity of 45%. The measurements of radishes started after the phase of three true leaves, and then dehydration started.

The volumetric water content (θ) was measured using a calibrated moisture meter ECH_2O with soil moisture sensor ECH_2O EC-5 (Decagon Devices, Pullman, USA). The measurement lasted five days, which was the dehydration time of the plants. It was measured once per day at the same time.

Measurement of photosynthetic parameters. The photochemical response at the Photosystem II level was analysed by a portable, battery-powered PAM fluorometer FluorPen FP 110 (Photon Systems Instruments, Drásov, Czech Republic) that enables quick and precise measurement of chlorophyll fluorescence parameters. For the evaluation of plants, we utilised selected parameters derived from the OJIP curve, including the maximum quantum yield of photosystem II (F_v/F_m), performance index (PI_{abs}), absorption flux per reaction centre (ABS/RC), a parameter expressing the rate of accumulation of closed reaction centres (MO), variable fluorescence at step I (V_i), and dissipated energy flux per reaction centre (DI_0/RC). To calculate fluorescence parameters reflecting the activity and efficiency of individual parts of the electron transport chain, we employed formulas derived from Strasser et al. (2000).

Plant growth parameters analysis. The dry weight of biomass of aboveground parts (leaves and bulbs) was measured. After ripening, the plants were removed from the soil, cleaned, weighed, and dried in an oven (2 days at 60 °C). After that, the dry biomass was weighed.

Statistical analyses. The effect of biochar application on soil properties was evaluated using a one-way analysis of variance (one-way ANOVA). Statistically significant effects at $P < 0.05$ were determined by the least significant difference (*LSD*) test. All analyses were performed in Statgraphics Centurion XV. I software (Statpoint Technologies, Inc., Warrenton, USA).

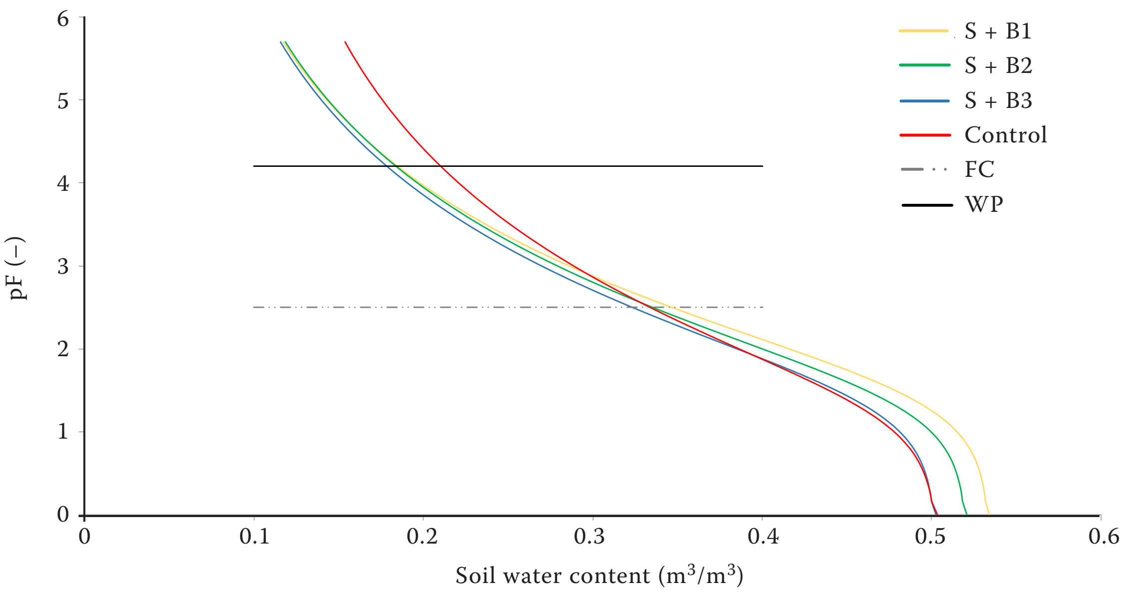

Figure 1. Soil water retention curves of pure soil (control) and its mixtures with biochar in comparison to soil water constants field capacity (FC) and wilting point (WP). S + B1 – soil + biochar < 125 µm; S + B2 – soil + biochar 125 µm – 2 mm; S + B3 – soil + biochar > 2 mm

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Table 2. Available water content for plants (AWC) for all studied variants

	Control	S + B1	S + B2	S + B3
	(m^3/m^3)			
AWC	0.126	0.165	0.154	0.147

S + B1 – soil + biochar < 125 μm; S + B2 – soil + biochar 125 μm – 2 mm; S + B3 – soil + biochar > 2 mm

RESULT AND DISCUSSION

Soil water retention. As shown in Figure 1, soil water retention curves differed for each treatment. The available water content for plants increased in all treatments with biochar (Table 2). The highest AWC value in comparison to the control was measured in the S + B1 treatment (about 31%). In S + B2 treatment, it was +23%; in S + B3 treatment, it was +17%. Results show that applying biochar into silt loam soil positively affects soil retention. Our results are consistent with other studies in the same area of research (Glab et al. 2016, Suliman et al. 2017, Duarte et al. 2019).

Soil water content during dehydration. At the outset of the measurement under optimal conditions, higher values of volumetric water content (θ) were observed, correlating with the fineness of the biochar grain size (Figure 2). Over the same duration, the values of (θ) decreased across all variants. That follows from the drought simulation. However, the soil moisture in all treatments consistently remained higher than that in the control throughout the entire process. This is in line with the theory that biochar particles have a positive impact on plant AWC.

On the fifth day of drought induction, the mean particle size of the biochar (S + B2) maintained the highest (θ) value (Figure 2). Liu et al. (2017) assert that biochar's increased intraporosity indicates that its intrapores can increase soil water storage. This inference is substantiated by statistical investigations conducted by Liu et al. (2017). Our experiment confirmed better water storage in the soil during simulated drought in the soil with added biochar but did not confirm that the larger the particles, the higher the water storage.

Plant biomass. Applying biochar to the soil had no significant effect on the amount of biomass in variant S + B1. However, it resulted in an increased yield for variants S + B2 and S + B3 compared to the control, but it was statistically not significant (Figure 3). The lower biomass yield observed in variant S + B1 and the control is probably due to reduced photosynthesis caused by water deficit stress. In the case of variants S + B2 and S + B3, this trend is not confirmed, which suggests that the larger size of the biochar particles could potentially retain water more effectively, thereby affecting both photosynthesis and plant biomass simultaneously. The lack of positive or potentially negative effects stemming from the smallest biochar particle dose could be ascribed to its heightened availability to the plant and the consequent impact on the soil's physical, chemical,

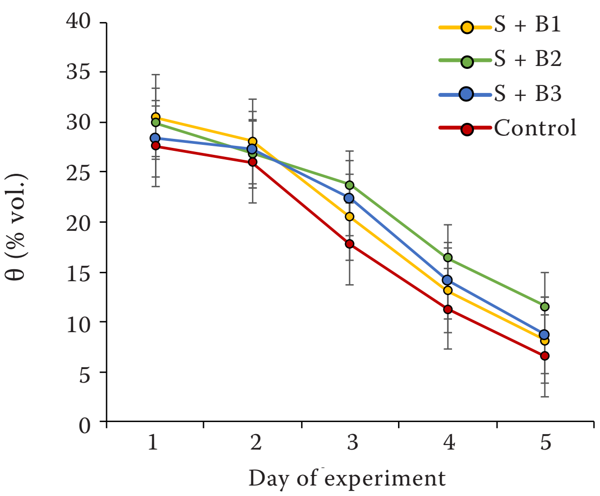

Figure 2. Comparison of average trends of the volumetric soil water content during time. Error bars represent standard errors (*n* = 5). S + B1 – soil + biochar < 125 μm; S + B2 – soil + biochar 125 μm – 2 mm; S + B3 – soil + biochar > 2 mm

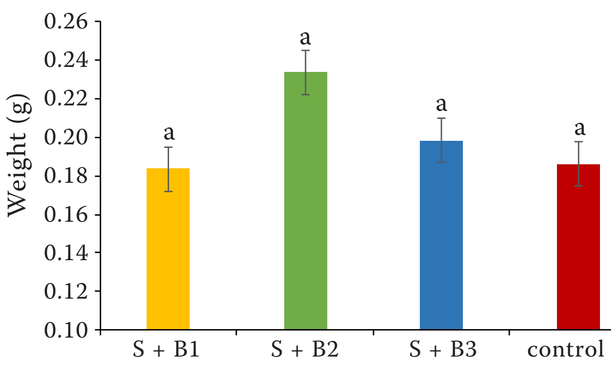

Figure 3. Comparison of the average dry biomass weight of five plants for each variant. Error bars represent standard errors (*n* = 5). Values with the same letter are not significantly different at $P < 0.05$ according to the least significant difference test (one-way ANOVA). S + B1 – soil + biochar < 125 μm; S + B2 – soil + biochar 125 μm – 2 mm; S + B3 – soil + biochar > 2 mm

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and biological properties. As Patwa et al. (2021) reported, drought stress reduced the leaves of plants due to the decrease in size, reduced production of new leaves, and the increase of their falling. They also concluded that the production and development of leaves are very sensitive to low water availability and, therefore, drought stress reduced biomass. In other words, at the size of biochar particle, plant response will result from the interactive effect of some direct (water contents and biochar) or indirect (the biochar supplied nutrients, biochar induced salinity, porosity and bulk density changes after biochar amendment, etc.) factors. The observed increase in plant growth in variants might be indicative of enhanced soil conditions. Zoghi et al. (2019) found that biochar enhances water infiltration within the root zone in clay loam, contributing to soil vitality and increased nutrient availability for plants. Uzoma et al. (2011) likewise reached the conclusion that biochar increased the available water capacity and saturated water content in sandy soils. Other investigators also indicated the positive effects of biochar on the fruit yield of cucumber (Solaiman et al. 2020), sunflower seed yield and oil production (Seleiman et al. 2019), and increase in maize growth and yield (Gholizadeh et al. 2020). Qian et al. (2019) observed a significant increase in the chlorophyll.

Chlorophyll fluorescence. The chlorophyll fluorescence technique offers a powerful way to explore photosynthesis efficiency, using chlorophyll fluorescence induction kinetics curves (OJIP) to capture transformations in the primary photochemical reaction process of PSII and the function of the photosynthetic mechanism (Lyu et al. 2016). F_v/F_m of the

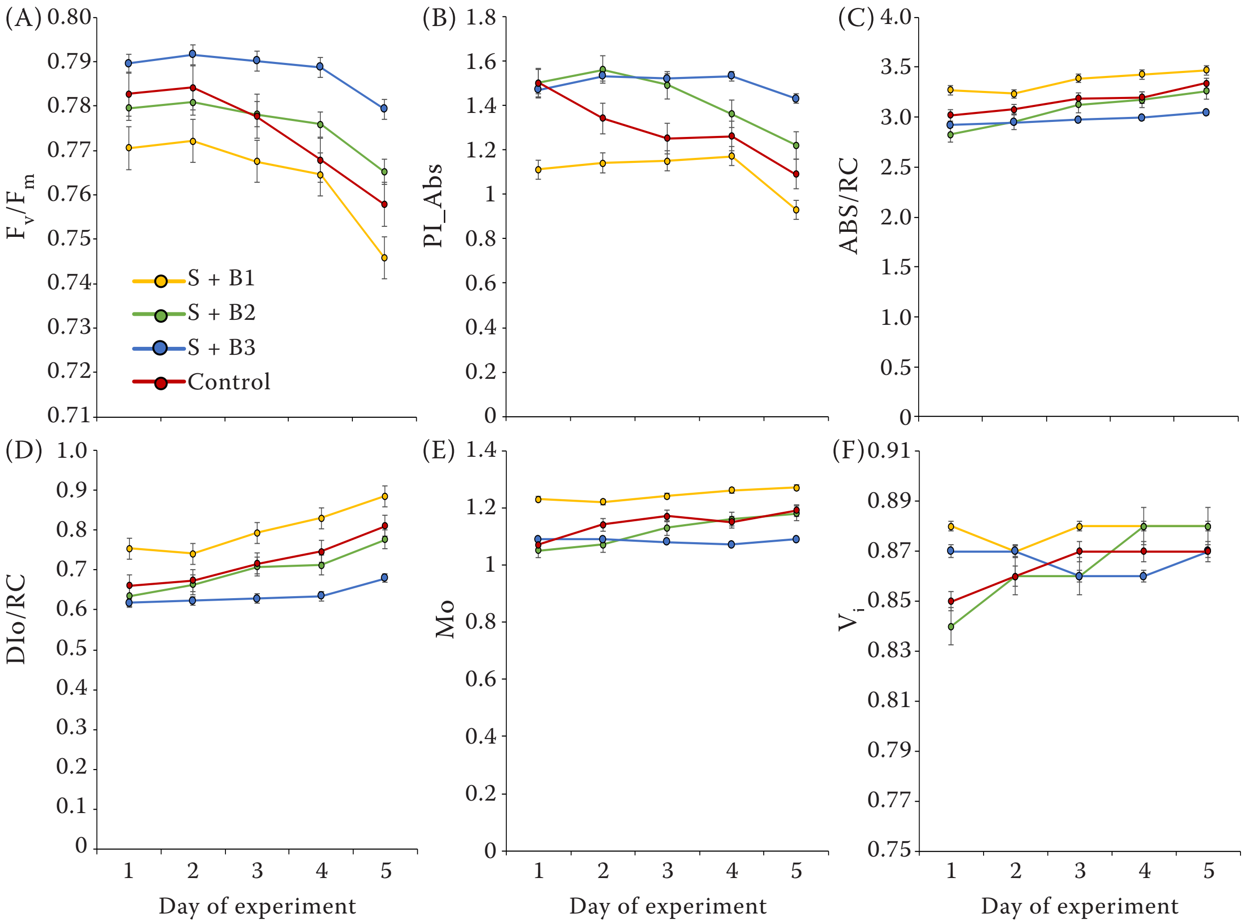

Figure 4. Average trends of selected parameters derived from measurements of fast fluorescence kinetics of chlorophyll on leaves expressed as averages, especially for individual variants of biochar particle size. (A) the maximum quantum yield of PSII photochemistry (F_v/F_m); (B) performance index (PI_Abs); (C) absorption flux per reaction centre (ABS/RC); (D) dissipated energy flux per reaction centre (DIo/RC); (E) a parameter expressing the rate of accumulation of closed reaction centres (Mo), and (F) the variable fluorescence at step I (V_i)

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control variant decreased sharply after drought stress after one day, while F_v/F_m of biochar treatment only began to decrease slowly (Figure 4) on the third day under drought stress. The sharp decrease came only on the fourth day after the beginning of the stress. This suggests that biochar treatment improves water management during drought stress. It also follows that the larger the size of the biochar particles, the better its soil water management.

The PI_{abs} can be a sensitive parameter in different crops and environmental stress conditions (Strasser et al. 2000). PI_{ab} behaved in two ways (Figure 4): (*i*) had a decreasing character for S + B2 and control and (*ii*) increased or maintained a trend until the third day and then sharply decreased for the S + B1 variant and decreased minimally for the S + B3 variant. Like F_v/F_m, however, he indicated that biochar treatment improves water management during drought stress. Liu et al. (2017) suggest that biochar with high pore volume and irregular shape will most effectively increase plant-available water in the soil. This was already confirmed for the θ parameter and the chlorophyll fluorescence measurement parameters, where both variants with larger biochar particles maintained more stable F_v/F_m parameter values than the control and the variant with small particles. The ABS/RC was high on the days of drought, and the value reached its maximum on day five (Figure 4). A similar trend was recorded in drought stress, significantly enhanced DI_0/RC. Strasser et al. (2000) suggest that drought-tolerant plants reduce the effective antenna size and absorb energy. Nevertheless, the drought-sensitive variant could not modulate the antenna size, leading to increased excitation pressure at the PSII reaction centres and consequent damage to the active reaction centres. The MO exhibited increment under drought stress for variant control, S + B1 and S + B2 (Figure 4). The V_i, which designates the variable fluorescence at step I, showed a higher increase in variant S + B1 than other variants (Figure 4).

In summary, biochar fraction size affects silt loam soil's water regime and cultivated radish's photosynthesis. Of the three analysed biochar fractions produced from willow, the best results were measured for variants S + B2 in silt loam soil. This study advances our understanding of how biochar particle size affects soil water retention and AWC with and without the plant's root system. It also underscores the potential of chlorophyll fluorescence techniques and biochar treatments to address challenges posed by environmental stresses and water scarcity in modern agriculture, with implications for soil management and sustainable agriculture.

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