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Biochar as drought management to enhance tolerance and recovery of sugarcane

Thi-Thuy-Hang Vu, Thi-Tuyet-Cham Le, Ngoc-Thang Vu*

Faculty of Agronomy, Vietnam National University of Agriculture, Hanoi, Vietnam

***Corresponding author: Ngoc-Thang Vu** ✉

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Abstract: Drought is becoming a serious constraint to sugarcane production, requiring efficient management strategies to mitigate its adverse effects. This study revealed effective use of biochar as a drought management strategy for sugarcane. The experiment was conducted using a completely randomized design with three biochar rates (0 - control, 5- and 10-tons ha^{-1}) under normal watering condition (control) and 4-week drought treatments during vegetative stage of sugarcane. Results showed that drought and biochar application significantly affected growth, physiological responses and recovery of sugarcane. During 1–4-week drought period, plant height, leaf number, and stalk diameter were significantly reduced compared to the control. A 4-week drought resulted in 10 – 50% reduction in leaf areas, root length and volume, and biomass relative to control. Drought reduced Fv/Fm and increased relative ion leakage and water saturation deficit. Sugarcane showed recovery ability after 4-week drought, with recovery ranging from 3% to 70% compared to the point prior stress released. Increasing biochar rate to 10 tons ha^{-1} had significantly positive effects on sugarcane growth and physiological responses under both normal and drought conditions. Biochar application also supported plant recovery, suggesting that biochar is an effective alternative strategy for mitigating adverse effects of drought.

Keywords: drought, vegetative stage, sugarcane, physiological traits, growth traits.

Introduction

With the increasing amount of drought-affected land worldwide, drought has become the significant environmental factor impacting plant growth. This is particularly true for water-intensive crops like sugarcane, where drought stress hinders growth and photosynthesis ability, ultimately reduces biomass and yield. Sugarcane is widely cultivated in tropical and subtropical regions, including countries like India, and Vietnam, across approximately 28.3 million hectares in nearly 90 countries, producing a total of 1.69 billion tons (Thibane et al., 2022). Drought can cause yield losses of 50 – 60% and has reduced global productivity by as much as 80% (Misra et al., 2022; Kumar et al., 2023; Tippayawat et al., 2023). In Vietnam, the largest sugarcane-growing regions are along central coast, where droughts often occur from early in the year through August (Bui et al., 2020). Drought stress affects all stages of sugarcane growth, of which, germination, tiller and grant vegetative growth stages are critical in sugarcane production and require careful water management (Ferreira et al., 2017).

The most common responses of sugarcane to drought stress include reduced stalk height, leaf rolling, crumbling and discoloration, reduced leaf area, stomatal closure, and leaf senescence (Inman-Bamber et al., 2012; Misra et al., 2020). Stomata closure minimizes water loss, acting as an adaptive response when drought begins (Saradadevi et al., 2017). However, these responses also impair leaf photochemistry and carbon metabolism, leading to decline in photosynthetic rate, sugar accumulation, and yield (McCormick et al., 2008; Zargar et al., 2017). The reduction in photosynthetic capacity is related to decrease in chlorophyll content (SPAD) and fluorescent (Fv/Fm) (Jangpromma et al., 2010; Dinh et al., 2019). Chlorophyll content decreased by 0.4 – 0.46 in drought-susceptible sugarcane varieties, compared to 0.66 - 0.69 in drought tolerant varieties when exposed to drought stress during tillering stage (Devi et al., 2018). Vasantha et al., (2005) reported decline in yield of 37% and in sugar yield of 43.88% in sugarcane subjected to drought stress. Recent studies have reported that biochar application can mitigate effects of drought stress by increasing soil water-holding capacity, thereby increasing water availability for plants, improving mineral absorption, and regulating stomatal conductance. Biochar significantly influences activity of reactive oxygen species (ROS) scavenging enzymes and provides an effective electron transfer mechanism to address toxic effects of ROS in plants (Mansoor et al., 2021). Biochar is recognized as a tool for improving crop productivity (Hasnain et al., 2022), water use efficiency (WUE), and tolerance to abiotic stress (Tang et al., 2022). Biochar application increases plant height and leaf area in crops subjected to drought stress, such as rice (Hazman et al., 2023), maize (Sattar et al., 2020), thereby improve plant growth. Hafez et al. (2020) showed that biochar application reduced electrolyte leakage and lipid peroxidation of stressed plants, which in turn enhanced membrane stability, relative water content, and water pressure under drought conditions. Given these benefits of biochar in managing various crops, especially under abiotic stress conditions, this study aims to investigate: (1) how drought affects the growth and physiological responses of sugarcane at vegetative stage; (2) how sugarcane recovers after stress is alleviated; and (3) the optimal biochar application rate for mitigating negative effects of drought stress on sugarcane.

Results

Growth responses of sugarcane under control and drought conditions with biochar application

ANOVA results revealed significant differences between drought treatment and biochar rates for plant height, number of leaves and stem diameter (Supplement Table 3). Increasing biochar rates increased sugarcane growth under both control and drought conditions. For example, at the rate of 10 tons ha^{-1} under normal watering conditions, stem diameter increased from 16.88 mm to 18.11 mm, 19.23 mm and 203.5 mm from week 1 to week 4. Under drought condition at the same biochar rate, stem diameter increased slightly from 16.85 mm to 17.17 mm at week 1 and 2, but then slightly decreased to 17.15 mm and 16.83 mm at week 3 and 4, respectively. An exception was observed in leaf number, which continued to increase slightly over 4-week drought period across all biochar rates.

Table 1. Leaf area, root volume, fresh above-ground and root weight and relative reduction (%) in sugarcane under biochar application of 0, 5, and 10 tons ha^{-1} and drought condition at 4-week drought compared with control.

Drought treatment	Biochar rates (tons ha^{-1})	Leaf areas (m^2) ± SD	Root length (cm) ± SD	Root volume (cm^3) ± SD	Fresh above-ground weight (g plant^{-1}) ± SD	Fresh root weight (g plant^{-1}) ± SD	Relative reduction (%) in comparison to control$^{\neq}$: Leaf areas	Root length	Root volume	Fresh above-ground weight	Fresh root weight
Control	0	9.23^{bc} ± 0.62	91.50^{b} ± 0.27	9.67^{c} ± 0.76	55.39^{c} ± 2.51	9.47^{bc} ± 0.41	-	-	-	-	-
	5	9.93^{ab} ± 0.57	93.6^{ab} ± 0.62	12.50^{b} ± 0.50	65.66^{b} ± 1.50	10.51^{b} ± 0.17	-	-	-	-	-
	10	10.97^{a} ± 0.73	96.0^{a} ± 0.53	14.67^{a} ± 0.58	70.34^{a} ± 0.78	12.99^{a} ± 0.67	-	-	-	-	-
Drought	0	6.62^{d} ± 0.41	71.7^{e} ± 0.4	8.50^{c} ± 0.50	30.93^{f} ± 1.14	4.79^{e} ± 0.31	-28.22^{B}	-21.64^{C}	-12.07^{A}	-44.17^{B}	-49.47^{B}
	5	8.23^{c} ± 0.31	80.0^{d} ± 1.4	10.0^{c} ± 0.50	37.48^{e} ± 0.24	6.19^{d} ± 0.42	-17.15^{AB}	-14.53^{B}	-20.0^{A}	-42.92^{AB}	-41.12^{AB}
	10	9.75^{abc} ± 0.67	86.38^{c} ± 1.91	13.0^{b} ± 0.5	42.59^{d} ± 1.45	8.45^{c} ± 0.47	-11.12^{A}	-10.02^{A}	-11.36^{A}	-39.45^{A}	-34.98^{A}
Means for drought treatment	Control	10.05 ± 0.94	93.70 ± 2.0	12.28 ± 2.24	63.80 ± 6.79	10.99 ± 1.62					
	Drought	8.20 ± 1.42	79.36 ± 6.49	10.50 ± 2.03	37.0 ± 5.15	6.47 ± 1.64					
		*	*	*	*	*					
Means for biochar rates	0	7.93^{C} ± 1.50	81.60^{C} ± 8.85	9.08^{C} ± 0.86	43.16^{C} ± 13.51	7.13^{C} ± 2.59					
	5	9.08^{B} ± 1.02	86.80^{B} ± 7.51	11.25^{B} ± 1.44	51.57^{B} ± 15.46	8.35^{B} ± 2.39					
	10	10.36^{A} ± 0.92	91.19^{A} ± 5.42	13.83^{A} ± 1.03	56.47^{A} ± 15.23	10.72^{A} ± 2.54					

Different lowercase letters show interaction significance among biochar rates and drought treatment conditions by Tukey's test at $p \leq 0.05$. Different capital letters show significance among biochar rates by Tukey's test at $p \leq 0.05$. * shows significance between drought treatment at $p \leq 0.05$. $^{\neq}$ Negative numbers indicate relative reduction.

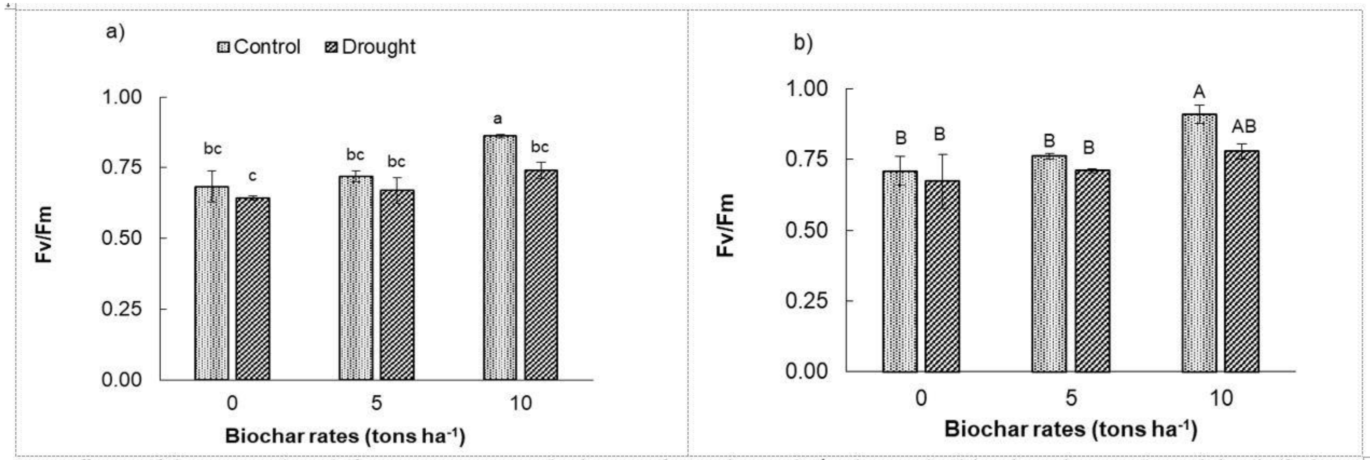

Fig 1. Quantum efficiency of photosystem II (Fv/Fm) of sugarcane in response to biochar rates of 0, 5, and 10 tons ha^{-1} under control and drought conditions on a) 4-week drought (final treatment day) and b) 10-day after re-watering. Vertical bars illustrate mean ± SD, n = 3. Different letters showed significance among biochar rates × drought treatment by Tukey's test at $p \leq 0.05$.

Table 2. Recovery of sugarcane for plant height, number of leaves and stem diameter and relative changes (%) in comparison to control under biochar application of 0, 5, and 10 tons ha^{-1} and under control and drought condition 2-week after re-watering

Drought treatment	Biochar rates (tons ha^{-1})	Plant height (cm) ± SD	Number of leaves ± SD	Stem diameter (mm) ± SD	Relative reduction (%) in comparison to control$^{\neq}$		
					Plant height	Number of leaves	Stalk diameter
Control	0	135.52^{c} ± 0.82	11.11^{c} ± 0.60	21.32^{b} ± 0.3	-	-	-
	5	138.22^{b} ± 2.11	12.0^{b} ± 0.01	22.04^{ab} ± 0.3	-	-	-
	10	144.67^{a} ± 1.52	13.78^{a} ± 0.44	22.73^{a} ± 0.78	-	-	-
Drought	0	125.48^{f} ± 1.5	9.56^{e} ± 0.53	15.50^{d} ± 0.43	-7.41^{a}	-14.0^{a}	-27.28^{b}
	5	128.39^{e} ± 1.25	10.33^{d} ± 0.50	16.88^{c} ± 0.75	-7.11^{a}	-13.89^{a}	-23.40^{a}
	10	131.04^{d} ± 1.31	12.0^{b} ± 0.71	17.53^{c} ± 0.45	-9.42^{b}	-12.9^{a}	-22.87^{a}
Means for drought treatment	Control	139.47 ± 4.19	12.3 ± 1.2	22.03 ± 0.77			
	Drought	128.3 ± 2.66	10.63 ± 1.18	16.64 ± 1.02			
		*	*	*			
Means for biochar application	0	130.50^{C} ± 5.3	10.33^{C} ± 0.97	18.41^{C} ± 3.01			
	5	133.31^{B} ± 5.33	11.17^{B} ± 0.92	19.46^{B} ± 2.71			
	10	137.86^{A} ± 7.14	12.89^{A} ± 1.08	20.13^{A} ± 2.75			

Different lowercase letters show interaction significance among biochar rates and drought treatment conditions by Tukey' test at $p \leq 0.05$. Different capital letters show significance among biochar rates by Tukey's test at $p \leq 0.05$. * shows significance between drought treatment at $p \leq 0.05$. $^{\neq}$ Negative numbers indicate relative reduction.

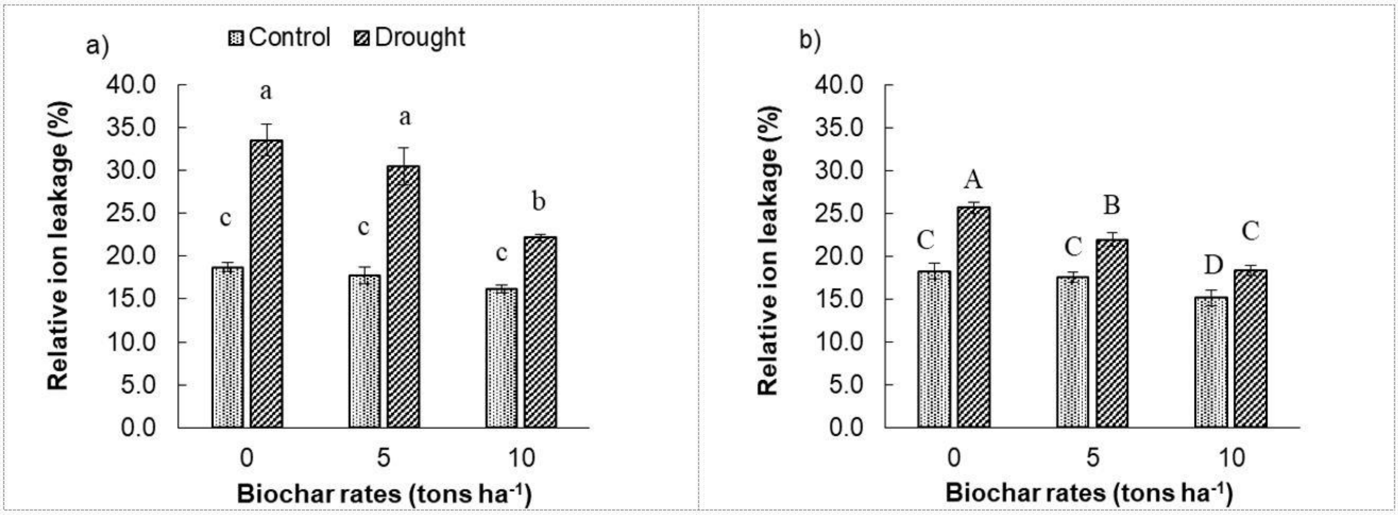

Fig 2. Relative ion leakage (%) of sugarcane in response to biochar rates of 0, 5, and 10 tons ha^{-1} under control and drought conditions on a) 4-week drought (final treatment day) and b) 10-day after re-watering. Vertical bars illustrate ± SD, n = 3. Different letters showed significance among biochar rates × drought treatment by Tukey's test at $p \leq 0.05$.

Leaf areas, root traits, and fresh above-ground weight under 4-week drought treatment were significantly lower than those under well-watered condition (Table 1). The average leaf area was significantly reduced under drought compared to normal conditions (8.20 m^2 vs 10.05 m^2). Means of fresh above-ground and root weights under drought were nearly half of those under control conditions (37.0 g plant^{-1} vs 63.80 g plant^{-1} and 6.47 g plant^{-1} vs 10.99 g plant^{-1}, respectively). Drought caused a relative reduction to the control in measured traits, ranging from 10.02 % (root length) to 49.47% (fresh root weight). Fresh weights of above-ground parts and root were more severed affected by drought than other traits, with relative reductions of about 34 – 49%.

Biochar application increased leaf areas, root growth and fresh weight under both control and drought treatments. Especially, increasing the rate to 10 tons ha^{-1} resulted in significantly higher sugarcane growth responses than at 0 and 5 tons ha^{-1}. For instance, the highest root length and volume were observed at 10 tons ha^{-1}, with 91.19 cm and 13.83 cm^3, respectively. Increasing biochar rates also enhanced plant responses to drought, resulting in higher values and lower relative reduction compared to control. After 4-week drought, root volume at 10 tons ha^{-1} was higher than at 5 tons ha^{-1} (13.0 cm^3 vs. 10.0 cm^3) and exhibited a significantly lower relative reduction (10.02% vs. 14.53%).

Growth recovery of sugarcane under control and drought conditions with biochar application

Sugarcane demonstrated recovery ability after 4-week drought for measured traits (Table 2, 3 & 4). Two weeks after the release of stress, means of plant height, number of leaves and stem diameter under drought were significantly lower than those under well-watered conditions, with values of 128.3 cm vs 139.47 cm, 10.63 leaves vs 12.3 leaves, and 16.64 mm vs 22.03 mm, respectively (Table 2). Although plants exhibited some recovery, relative reduction in growth compared to the control ranged from 7.11 to 27.28%, indicating severe effects of drought. However, biochar application mitigated drought effects. For instance, mean of plant height at 10 tons ha^{-1} was 137.86 cm, followed by 133.31 cm at 5 tons ha^{-1} and 130.50 cm with non-biochar application.

Similarly, leaf areas, root traits, and fresh above-ground weight increased after drought released. Higher biochar rates led to significant recovery. Under drought treatment, leaf areas recovered to 11.69 cm^2, 9.85 cm^2 and 7.32 cm^2 at biochar rates of 10, 5 and 0 tons ha^{-1}, respectively (Table 3). Root length, root volume, fresh above-ground, and fresh root weight reached their highest levels at 10 tons ha^{-1}, corresponding to 95.30 cm, 13.77 cm^3, 67.05 g plant^{-1} and 11.64 g plant^{-1}, respectively. The relative increase compared to the point before re-watering ranged from 3.30% to 68.94% (Table 4). Overall, fresh weights of above-ground parts and roots had significant high relative increases compared

Table 3. Recovery of sugarcane for leaf areas, root length, root volume, fresh above-ground and root weights under biochar application of 0, 5, and 10 tons ha^{-1} and under control and drought condition 2-week after re-watering

Drought treatment	Biochar rates (tons ha^{-1})	Leaf areas (m^2) ± SD	Root length (cm) ± SD	Root volume (cm^3) ± SD	Fresh above-ground weight (g plant^{-1}) ± SD	Fresh root weight (g plant^{-1}) ± SD
Control	0	12.75^{bc} ± 0.81	99.23^{b} ± 1.60	11.17^{d} ± 1.04	83.16^{c} ± 0.16	14.43^{c} ± 0.19
	5	13.46^{b} ± 0.3	104.87^{a} ± 1.56	15.83^{b} ± 0.76	88.82^{b} ± 0.86	15.73^{b} ± 0.48
	10	15.33^{a} ± 0.88	107.73^{a} ± 1.86	17.93^{a} ± 0.90	104.73^{a} ± 0.19	17.74^{a} ± 0.75
Drought	0	7.32^{e} ± 0.53	74.07^{d} ± 0.38	9.03^{d} ± 0.46	46.40^{f} ± 2.08	8.09^{e} ± 0.41
	5	9.85^{d} ± 0.60	85.08^{c} ± 1.08	10.33^{d} ± 0.58	52.31^{e} ± 0.4	9.31^{e} ± 0.20
	10	11.69^{c} ± 0.38	95.30^{b} ± 1.90	13.77^{c} ± 0.25	67.05^{d} ± 0.82	11.64^{d} ± 0.52
Means for drought treatment	Control	13.84 ± 1.31	103.94 ± 4.02	14.98 ± 3.10	92.24^{a} ± 9.69	15.97 ± 1.51
	Drought	9.62 ± 1.95	84.82 ± 9.26	11.12 ± 2.07	55.25^{b} ± 9.28	9.68 ± 1.60
		*	*	*	*	*
Means for biochar application	0	10.03^{C} ± 3.03	86.65^{C} ± 13.82	10.22^{C} ± 1.27	64.78^{C} ± 20.18	11.26^{C} ± 3.49
	5	11.65^{B} ± 2.03	94.97^{B} ± 10.9	13.08^{B} ± 3.07	70.57^{B} ± 20.01	12.52^{B} ± 3.53
	10	13.51^{A} ± 2.08	101.52^{A} ± 7.01	15.85^{A} ± 2.36	85.89^{A} ± 20.65	14.69^{A} ± 3.39

Different lowercase letters show interaction significance among biochar rates and drought treatment conditions by Tukey's test at $p \leq 0.05$. Different capital letters show significance among biochar rates by Tukey' test at $p \leq 0.05$. * shows significance between drought treatment at $p \leq 0.05$.

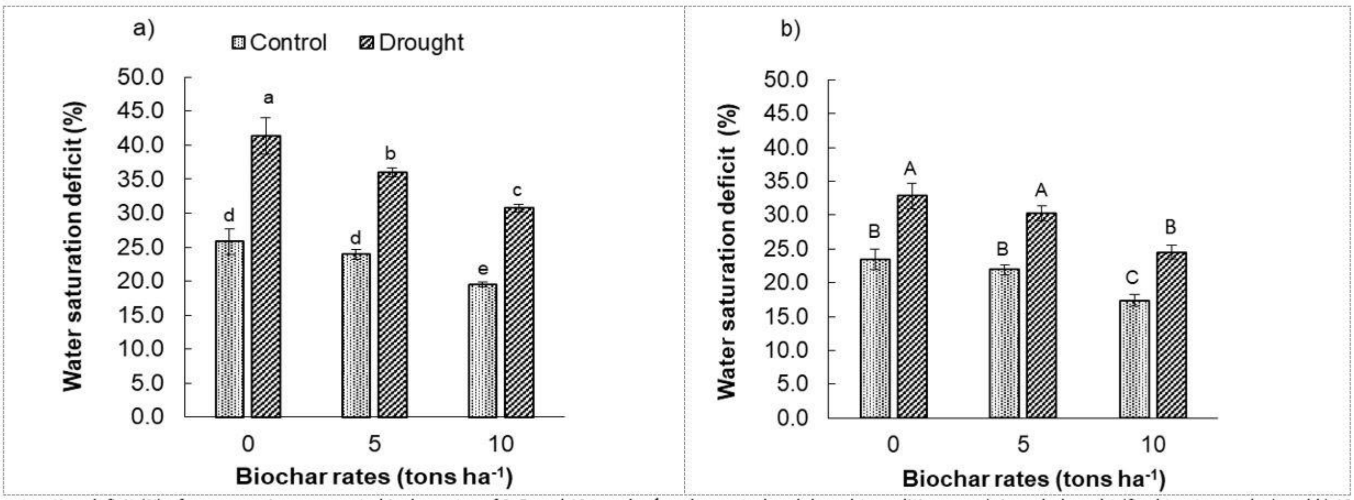

Fig 3. Water saturation deficit (%) of sugarcane in response to biochar rates of 0, 5, and 10 tons ha^{-1} under control and drought conditions on a) 4-week drought (final treatment day) and b) 10-day after re-watering. Vertical bars illustrate ± SD, n = 3. Different letters showed significance among biochar rates × drought treatment by Tukey's test at $p \leq 0.05$.

to the point prior to re-watering (~ 40 – 70%), followed by leaf areas (~ 10 - 20%), root length and volume (~ 3 – 10%). When compared to control, the relative reduction was significantly lower at the biochar rate of 10 tons ha^{-1}. For example, the relative reduction in root length was 11.54%, in fresh above-ground weight was 35.98%, and in fresh root weight was 34.37%.

Physiological responses and recovery of sugarcane under control and drought condition with biochar application

In terms of Fv/Fm, application of 10 tons ha^{-1} resulted in significantly better performance than 0 and 5 tons ha^{-1} under both 4-week drought and 10-day recovery (Fig. 1). Especially, Fv/Fm of sugarcane recovered to a level that was not significantly different from the control at the rate of 10 tons ha^{-1} (0.91 vs 0.78) (Fig. 1b). Drought also caused significantly high relative ion leakage, ranging from 22.15 – 33.49 %, compared to 16.15% to 18.67% under control (Fig. 2). The highest relative ion leakage was observed with non-biochar application, reaching 33.49% at final treatment day and 25.63% at 10-day after rewatering. In contrast, increasing biochar rate to 10 tons ha^{-1} significantly reduced the relative ion leakage to 22.15% and 18.31%, respectively.

Water saturation deficit was higher under drought conditions compared to normal watering. Water saturation deficit in sugarcane at 4-week drought ranged from 30.79% to 41.33%, compared to 19.55% to 25.82% under control conditions (Fig. 3a). At 10-day recovery, water saturation deficit in drought-exposed sugarcane reduced to 24.52 – 32.84% but remained higher than the control range of 17.38% to 23.45% (Fig. 3b). Application of 10 tons ha^{-1} resulted in the lowest water saturation deficit, with 30.79% at final treatment day and 24.52% at 10-day recovery.

Discussions

The adverse effects of drought on growth, physiological, and recovery of sugarcane at the vegetative stage were observed. The longer exposure to drought, the more severe sugarcane was affected. Drought caused growth reduction in sugarcane by 10 – 50% compared to well-watered conditions, depending on growth characters, with high relative reduction seen in fresh weights of above-ground plant parts and roots, which ranged from ~ 35 – 50% (Table 1). Water deficit is a critical factor during early stages of sugarcane growth and causes changes in morphology, agronomic parameters (e.g., leaf area, stalk diameter, plant height, root characters), and yield-determining characteristics (Jaiphong et al., 2016; Ryes et al., 2021). Various studies have reported similar reduction in agronomic traits under drought, such as 29.2% in leaf area, and 7.4% in stalk diameter (Misra et al., 2020), and 15 - 54% in plant height (Wagih et al., 2003; Hemaprabha et al., 2004; Endres et al., 2018). Consistent with this study, stalk weight under drought has shown significant reductions compared to normal conditions, with a range varying from 21.3% (Misra et al., 2020), to 25.5 – 28.6% (Hemaprabha et al., 2013; Khaled et al., 2018) and up to 64.2% (Hemaprabha et al., 2004). The root weight reduction observed in this study (35 – 50%) was higher than reported in previous studies, such as 17% reduction noted by Misra et al. (2020).

Physiological responses to drought involve various processes such as photosynthesis, water use efficiency, oxidative stress

Table 4. Relative changes of leaf areas, root length, root volume, fresh above-ground and root weights in sugarcane under biochar application of 0, 5, and 10 tons ha⁻¹ and drought in comparison to point prior re-watering and control 2-week after re-watering

Biochar rates (tons ha⁻¹)	Relative changes (%) in comparison to point prior re-watering≠					Relative reduction (%) in comparison to control≠				
	Leaf areas	Root length	Root volume	Fresh above-ground weight	Fresh root weight	Leaf areas	Root length	Root volume	Fresh above-ground weight	Fresh root weight
0	10.52ᵃ	3.30ᵇ	6.27ᵃ	50.04ᵃ	68.94ᵃ	-42.55ᵇ	-25.36ᶜ	-17.01ᵃ	-44.20ᵇ	-43.96ᵇ
5	19.63ᵃ	6.35ᵇ	5.90ᵃ	39.57ᵇ	50.35ᵇ	-26.87ᵃ	-18.87ᵇ	-34.74ᵇ	-41.11ᵇ	-40.82ᵇ
10	19.84ᵃ	10.33ᵃ	3.33ᵃ	57.42ᵃ	37.85ᵇ	-23.73ᵃ	-11.54ᵃ	-13.05ᵃ	-35.98ᵃ	-34.37ᵃ

Different lowercase letters show significance by Tukey's test at $p \leq 0.05$. ≠ Positive and negative numbers indicate relative increase and relative reduction, respectively

mitigation, nutrient absorption and uptake (Cha-um et al., 2012; Ryes et al., 2021). This study also analyzed changes in physiological traits of sugarcane under drought, including quantum efficiency of photosystem II (Fig. 1), relative ion leakage (Fig. 2) and water saturation deficit (Fig. 3). Often, photosynthesis is negatively affected by water-limiting conditions. Photosynthesis rate decreases due to structural damage of photosynthetic systems (Gomathi et al., 2011), reduced stomatal conductance, and chlorophyll content reduction (Khonghintaisong et al., 2018; Dinh et al., 2019). Additionally, maximum efficiency of photosystem II (PSII) (Fv/FM), which measures yield of chlorophyll fluorescence, also reduces under drought condition, with reductions ranging from 1.2 to 21.2% (Cha-um et al., 2012; Leanasawat et al., 2021). Drought-sensitive genotypes often showed a significant decline in Fv/Fm (Kaur et al., 2015; Devi et al., 2018). In this study, Fv/Fm of sugarcane under well-water ranged from 0.68 to 0.91, reduced to 0.64 – 0.74 under drought, and recovered to 0.67 – 0.78 after drought was released (Fig. 1). These Fv/Fm values under drought stress were higher than those reported in other studies, which revealed values of 0.36 – 0.46 for susceptible and 0.60 – 0.69 for tolerant varieties (Graca et al., 2010; Devi et al., 2018). Songsri et al. (2019) reported Fv/Fm values of 0.78 and 0.81 during recovery. The higher Fv/Fm values observed in the sugarcane cultivar used in this study align with its classification as drought-tolerant. Furthermore, biochar application positively affected maintenance of Fv/Fm within the drought-tolerance range, thereby supporting plant's ability to endure adverse conditions. In contrast to reduction in Fv/Fm, relative ion leakage and water saturate deficit increased as drought stress was applied (Fig. 2a & 3a).

In this study, sugarcane exhibited recovery ability in both growth and physiological traits after 4-week drought. Plants showed varying degrees of growth recovery, ranging from ~ 3% to 70% compared to the point before stress was alleviated, with fresh weights of above-ground parts and roots displaying the most significant recovery (35 -70%) (Table 4). Physiological recovery was observed at 10-day, with increases in Fv/Fm and decreases in relative ion leakage and water saturation deficit (Fig. 1b, 2b & 3b).

Other studies have applied similar or even longer drought duration to assess responses and recovery of different sugarcane genotypes. For instance, drought duration have ranged from 15 to 30 days under glasshouse conditions (Jaiphong et al., 2016; Bui et al., 2020), to 72 days (Leanasawat et al., 2021) and 120 days (Khonghintaisong et al., 2021) in field conditions. Tippayawat et al. (2023) showed differential responses in leaf gas exchange, chlorophyll fluorescence, leaf chlorophyll, relative water contents, biomass and leaf area index when plant cane and ratoon cane were imposed short- and long-term drought of 3- and 5-months of water-withholding, respectively. Although sugarcane, with its 12-month growth duration, has its mechanism to cope with and recover from drought, the vegetative growth phase is crucial and highly susceptible (Silva et al., 2007; Machado et al., 2009; Dlamini, 2021). Prolonged drought during these stages can lead to reduction in sucrose content and juice purity, as plant uses stored sucrose for metabolism (Begum et al., 2012). Consequently, both yield and quality of sugarcane are significantly reduced (Dlamini, 2021).

Various management practices have been studied to minimize adverse effects of drought on sugarcane, including supplementary irrigation, water reservoirs (Singels et al., 2016; Singels et al., 2019), and mulching (Ramburan and Nxumalo, 2017). However, these practices often result in increased farming operational costs or are constrained by practical, economical concerns, and undesirable plant growth responses (Singels et al., 2016; Ramburan and Nxumalo, 2017; Dlamini, 2021). Recently, biochar application has been shown to alleviate effects of abiotic stresses in many plants at different growth stages (Hasnain et al., 2022; Wu et al., 2023). Biochar has mitigated drought stress in rice (Hazman et al., 2023), maize (Sattar et al., 2020), and both drought and salinity in soybean (Zhang et al., 2020) and tomato seedlings (Zhang et al., 2023).

Use of biochar as a measure to minimize adverse effects of drought on growth and physiological responses of sugarcane at the vegetative stage was also observed. Firstly, these findings align with previous research in aspect that biochar application generally promotes plant growth and favours physiological responses (Hasnain et al., 2022; Hazman et al., 2023). Secondly, under stress conditions such as drought, biochar enhanced tolerance of sugarcane, leading to significantly higher growth responses and lower relative reductions compared to conditions without biochar application. Increasing biochar rates, particularly at 10 tons ha⁻¹, further improved growth and physiological responses to drought stress in sugarcane. Recent biochar-based fertilizer, such as biochar-compost mixture, also offers an alternative utilization of biochar to enhance drought tolerance in plants grown in low-fertile soils (Hazman et al., 2023).

Materials and methods

Plant materials

Plants of ROC10 sugarcane variety at six-month-old stage were used as propagation material.

Experimental culture

Twenty-five-day-old seedlings were transplanted into plastic pots (30 cm diameter × 25 cm height) filled with 10 kg of alluvial soil. Experimental soil properties are shown in Supplement Table 1. The Oakwood biochar with properties reported by Rajapaksha et al. (2019) (Supplement Table 2). Modified Hoagland's nutrient solution as described in Vu et al. (2023) was used to water plants weekly with 200 mL one week after transplanting.

Experimental design

A completely randomized experimental design was adopted with three replications (10 pots per replication for each treatment). Biochar rate was the main factor, consisting of three application rates (0, 5, and 10 tons ha⁻¹, equivalent to 0, 35.33 g, and 70.65 g per pot, respectively), and water stress condition was the sub-factor (non-water stress and water stress conditions).

To prepare pots, they were first saturated with water, allowed to drain overnight, and then weighed to determine water-holding capacity of each pot. This measurement served as basis for regular watering (control) and for imposing drought stress. Before imposing drought stress (two months after transplanting), pots were again saturated with water, after which watering was withheld for 4 weeks, until plants showed wilting signs, and the available water was depleted. After 4-week drought, sugarcane plants were rewatered to assess recovery.

Growth measurement

Growth responses to drought were measured over 4-week drought period and 2 weeks after recovery. Specifically, plant height, number leaves and stem diameter were measured weekly during 4-week drought and at 2 weeks after re-watering. Leaf areas, root length, root volume, fresh above-ground weight, and fresh root weight were measured at the end of 4-week drought and 2 weeks after re-watering. Leaf area was determined using a leaf area meter (Delta-T Device Ltd., Burwell, Cambridge, UK). Root volume was determined using the volume displacement technique as described by Burdett (1979).

Physiological measurement

Quantum efficiency of photosystem II (Fv/Fm), relative ion leakage, water saturation deficit in leaves were recorded on final treatment day of drought stress (after 4-week drought) and on 10-day after re-watering. The quantum efficiency was determined using a modulated fluorometer (Opti-Sciences, Hudson, USA) after 30 minutes of dark adaptation on the second top visible leaf from 9:00 to 11:00 am, on the same leaf of 3 plants. Relative ion leakage was measured as the leakage of electrolytes from leaves of 3 plants of similar size using a conductivity meter (AG 8603, SevenEasy, Mettler Toledo, Switzerland), as described by Vu et al. (2023). In brief, leaf segments (~ 1 cm^2) were harvested, washed, blotted dry, weighed, and then placed in stopped vials filled with an exact volume of deionized water. The stopped vials were then incubated for 2 hrs. in darkness with continuous shaking before heated at 80℃ for 2 hrs. to measure conductivities C1 and C2, respectively. Relative ion leakage was calculated as follows (Zhao et al., 2007):

$$\text{Relative ion leakage (\%)} = \frac{C1}{C2} \times 100$$

Leaf water saturation deficit (WSD) was determined using 1 cm^2 leaf segments as described in Slavík (1963):

$$\text{WSD (\%)} = \frac{FM1 - FM0}{FM1 - DM} \times 100$$

Where FM0 and FM1 were initial fresh mass and mass of leaf segment under treatment and fully water-saturated conditions, respectively. DM was dry mass of the same leaf segment.

Data analysis

Growth parameters were gathered from randomly selected 9 plants per treatment for statistical analysis. For physiological parameters (e.g., leaf quantum efficiency of photosystem II (Fv/Fm), relative ion leakage, and water saturation deficit), 3 plants per treatment were randomly selected for statistical analysis. Data were analyzed using ANOVA in Minitab ver 20.1. Multiple mean comparisons were conducted using Tukey's test at $p \leq 0.05$.

Conclusions

The study showed that sugarcane growth and physiological responses were adversely affected by drought stress at the vegetative stage, with relative reductions in growth traits observed during both drought and recovery periods. Biochar application significantly increased plant growth, and Fv/Fm under both non-drought and drought conditions. Biochar treatment decreased leaf water saturation deficit and relative ion leakage in both conditions. Biochar utilization is an effective measure to mitigate negative effects of drought stress on sugarcane at the vegetative stage, with a recommended application rate of 10 tons ha^{-1}. However, further study is needed to investigate impacts of biochar on sugarcane growth at different stages under drought stress in the field, particularly in combination with other fertilizers, such as compost, to increase both yield and economical efficiency for sugarcane growers.

Conflict of Interest: Authors declare no conflict of interest.

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