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Effects of Watering Regime and Biochar on the Growth, Biochemical Constituents and Drought Susceptibility of Seedlings of Some Urban Forest Tree Species



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Abstract

Background: Climate stress and soil constraints of urban landscapes would limit establishment, shade effectiveness and ecosystem services of urban forests. Developments of the plant components of urban green infrastructure must identify tree species that are well adapted to climate stress factors. The effects of effects of wet-dry cycle and biochar on the growth and physiological attributes, biochemical constituents, and drought susceptibility of seedlings of some urban forest tree species (UFTS) was evaluated in the nursery.

Methods: The tested UFTS were Bauhinia monandra, Delonix regia, Terminalia catappa, Dypsis lutescens and Veitchia merrillii. Seedlings were subjected to 80 % field capacity (FC) watering and dry down treatment, and biochar amendment or not. Measured growth physiological and biochemical variables of UFTS seedlings were deployed for ranking the evaluated UFTS for drought susceptibility.

Results: Relative to well watering, dry down treatment produced lower values of root and shoot biomass, leaf area, relative water content, net assimilation rates, leaf chlorophyll and carotenoids contents. Dry down treatment enhanced accumulation of proline, soluble sugars, flavonoids and crude protein contents and elicited changes in enzymatic activities. Biochar amendment of dry down treatment enhanced growth, physiological and biochemical attributes of UFTS seedlings and induced enzymatic antioxidant activities. The increasing trends of drought tolerance among the UFTS were B. monandra, D. regia, T. catappa, D. lutescens and V. merrillii for both biochar amended and non-amended dry down condition.

Conclusion: Study identified drought susceptibility of evaluated UFTS seedlings and relevance of biochar for moisture deficit stress alleviation

Keywords: Urban Green Infrastructure; Antioxidant; Osmotic Adjustment; Enzymatic Activites; Moisture Deficit; Amendment

Abbreviations: ROS: Reactive Oxygen Species; UFTS: Urban Forest Tree Species; DSI: Drought Susceptibility Indices; HSD: Honest Significant Difference; TSS: Total Soluble Sugar; ASC: Ascorbic Acid; DSI: Drought susceptibility index

Introduction

Global environments change manifests as climate change and extreme weather events. Climate change may increase pressures on water resources, water stress situation and high temperatures with attendant shifts in environment boundaries [1,2]. Extreme temperatures will result in lethal heatwaves and urban heat island (UHI) phenomenon with consequences for human thermal comfort and heat related illnesses across the world. It is important to promote development of urban forestry into urban landscape development for building resilience to climate stress Roll of et al., 2009 [3,4]. Benefits of urban vegetation (urban green infrastructure) are attributable to tree modulation of solar radiation properties, evaporative cooling, carbon sequestration, water cycling and biodiversity conservation [5-7]. Climate stress and soil constraints of urban landscapes affect phenology and physiological functions, shade effectiveness, and ecosystem services of urban park and street trees [8,3,7]. In order to optimize the benefits of urban forestry, it is necessary to identify adaptable tree species to climate stress now and under future climate scenarios.

Strategies for abiotic stress tolerance in plants may involve complex, interacting mechanisms of. desiccation tolerance and drought performance [9,10] Mannan et al. 2016, [11]. In plants, water deficit stress may alter metabolic pathways, morphological, physiological, ultra-structural, biochemical features [12] Zhang et al. 2013, [13,14]. The effects of drought on msorphological and physiological traits and compatible metabolites and osmolytes of annual and perennial species has been evaluated severally [15-17]. Limitations in soil moisture status affect phytochemicals such as total soluble solids, organic acids, and soluble carbohydrates Keller & Ludlow, 1993, Khan et al., 2015, [18-20] and Soni et al. (2015). Changes in chlorophyll and carotenoid contents have been implicated as index of plant response to drought stress Pastori and Trippi, 1992, [21], and functions to protect against damage to chloroplasts caused by active oxygen species [22,23]. Increases in proline accumulation in leaves of water-stressed plants have been reported [24,23]. This is attributable to proline's role as a source of respiratory energy in addition to changes in the concept of free proline and total soluble sugars in water-stressed leaves [25,26].

Water stress is known to trigger excitation of photosynthetic pigments leading to accumulation of reactive oxygen species (ROS) such as singlet oxygen (O), superoxide anions (-O2-), peroxide (-O2-2), hydroxyl ion (HO-) and hydroxyl radical (OH-) in the chloroplasts. Reactive oxygen species can damage phospholipids of cell membrane and increase lipid peroxidation measured as Malondialdehyde (MDA) Mittler, 2002; Moller et al., 2007, [27]. In curtailing the impacts of oxidative stress, plants have developed complex enzymatic system involving reactive oxygen species (ROS), scavenging enzymes (superoxide dismutase, catalase; ascorbate peroxidase, Glutathione reductase etc.) and non-enzymatic antioxidants (glutathione, ascorbic acid, carotenoids) [28-30]. Urban landscapes exhibit specific microclimate and soil characteristics different from undisturbed ecosystems Degaetano, 2000, Rollof et al., 2009, [5].

Organic amendment, in particular, the use of biochar has been suggested as a viable tool to improve productivity, aesthetic performance, and carbon sequestration of urban forest species [31-34]. Biochar is a fine-grained and porous substance generated via pyrolysis of biomass-derived feed stocks under oxygen-limited conditions [35-36]. Biochar impacts soil properties: hydraulics, microbial activities, decomposition of native organic carbon content [38,35,39]. Soil amendment using biochar is considered as a means to mitigate impacts of water deficits on plants and for enhancing water use efficiency (Liang et al., considered as al., 2010, Eyles et al., 2015) [40-42]. The aim of this study is to evaluate the effects of watering regime and biochar amendment on the physiological attributes, biochemical constituents of seedlings of some urban forest tree species (UFTS) in the nursery.

Materials and Methods

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The study was carried out in the screenhouse of Wesley University, Ondo, Nigeria (Geo-coordinates of: 7.100005,

4.841694; DMS Lat. 7º6'0.0180"N; DMS Long. 4º50'30.0984"E, 387 m above sea level in a rainforest zone of southern Nigeria. Seedlings of urban forest trees species namely, Bauhinia monandra Kurz (Fabaceae), Delonix regia (Bojer ex Hook) Raffin (Fabaceae), Terminalia catappa L. (Combretaceae), Dypsis lutescens (H. Wendl.) Beentze & J. Dransf. (Arecaceae) and Veitchia merrillii (Becc.) H. E. Moore (Arecaceae) were raised by sowing seeds in plastic pots (Upper × Lower diameter × Height = 26 × 20 × 30 cm) containing 6 kg of top garden soil. Seedlings were watered to pot capacity for one month to allow acclimatization and thereafter subjected to well watering (80 % field capacity: FC) and dry down treatements, and sawdust biochar amendment or not. Dry down treatment was imposed by water withholding from potted UFTS seedlings for 42 days (6 weeks) followed by re-watering after treatment had induced significant amount of leaf abscission. The measured UFTS variables were deployed to compute species drought susceptibility indices (DSI) in order to rank the aesthetic performance and species tolerance of soil moisture deficit stress. For each species and treatment, 30 pots with one seedling each were maintained. Measurement of morphological and physiological traits and biochemical constituents were carried out monthly and average of the pooled data were subjected to statistical analysis for comparison of growth attributes and drought susceptibility of each tested species. Stem height and girth, number of leaves per plant, root length and ratio of root to shoot were measured using standard methods. Plant leaf area was determined using Pearcy et al., (1989) and total leaf area was estimated as the product of leaf area and number of leaves per plant. Biomass (dried at 80 ± 2°C) of leaf, stem and roots was recorded by harvesting three seedlings from each treatment. Photosynthetic pigment (chlorophyll a, b and total chlorophyll and carotenoids) and relative water contents were estimated using Weatherley (1950) and Lichtenthaller (1987) formulas respectively.

Estimation of Biochemical Constituents and Enzymatic antioxidants activities in UFTS

Contents of proline, soluble sugar (TSS), ascorbic acid (Asc) and phenolic acid (TPC) on fresh weight basis were estimated according to the procedures of [43,44] AOAC (2000) and [45] respectively. Peroxidation of membranes lipid measured as malondialdehyde (MDA) content was determined by following the methods of Hodges et al., (1999). For assessment of activities of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPx), the procedures of Giannopolitis and Ries (1977), Aebi and Lester (1984) and Chance and Maehly (1955) were adopted.

Drought Susceptibility of UFTS Seedlings

The drought susceptibility index (DSI) measured in percentages was calculated as the ratio of some measured morphological, physiological, and biochemical attributes of urban tree species under well-watered (X2) and water stress conditions (X1) as. Drought Susceptability Index (DSI) (%) = $100 \times \frac{\text{measured Traits under Drought Stress (X1)}}{\text{measured Traits in control Condition (X2)}}$ Statistical analysis of data

Data obtained on measured variables of the UFTS seedlings were subjected to two way analysis of variance (ANOVA) while the significant means was separated using the Tukey's Honest Significant Difference (HSD) test at P < 0.05.

Results

The treatments (well watering and dry down treatments and biochar application) affected the growth, physiology and biochemical constituents and drought susceptibility of seedlings of tested urban forest species. Among the seedlings, differences were observed between well watering and dry down condition for stem height, leaf area, root and stem and leaf biomass. Differences observed were significant for number of leaves and shoot weights between well watering and dry down treatments (Table 1). Biochar amendment of dry down treatment enhanced almost all growth variables of UFTS seedlings as well as physiological attributes and biochemical constituents of seedlings of urban forest species. For both biochar amended and non-amended dry down treatments, leaf relative water content (RWC) of B. monandra, D. regia, T. catappa, D. lutescens and V. merrillii seedlings substantially (P<.05) reduced compared to values under well watering (Table 2). The inclusion of biochar to dry down induced increases of ~ 17.0, 2.4, 10.8, 7.8 and 5.3 % in leaf RWC of seedlings respectively compared with dry down without biochar amendment.

Table 1: Effects of watering regime ar	nd biochar on the growth	attributes of seedlings of urban	forestry tree species (UFTS).
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Species	Trts	SH	LN	SG	LA	RL	RFW	SFW	LFW	WPFW
		cm		cm	cm ²	cm	g	g	g	g
	WW	17.656a	8.222a	1.628a	49.317a	16.694a	3.165a	3.360a	3.324a	9.849a
	DC	15.022a	3.278c	1.378b	42.903b	17.089a	3.207a	2.712b	2.095b	8.015a
B. monandra	DCB	16.133a	6.389b	1.556a	47.316a	16.972a	3.178a	3.189ab	2.832a	9.199a
	WW	13.472a	3.033a	1.027a	12.478a	12.372a	1.365a	1.773a	1.104a	4.242a
D. lutescens	DC	12.656a	2.567b	0.944b	10.370a	12.456a	1.469a	1.708a	0.954a	4.131a
	DCB	13.233a	2.817ab	0.962b	11.637a	12.428a	1.454a	1.751a	1.080a	4.286a
	WW	19.011a	5.950c	3.006a	182.770a	21.117a	4.006a	3.952a	2.766a	10.72a
D. regia	DC	14.433b	2.928a	2.839a	150.407b	21.372a	4.423a	2.986b	1.633c	9.043a
	DCB	16.456ab	4.297b	2.928a	170.847ab	21.233a	4.190a	3.541ab	2.256b	9.987a
	WW	17.928a	9.389a	2.339a	78.411a	18.306a	3.578a	4.511a	3.739a	11.829a
T. catappa	DC	13.467b	4.889b	2.233a	71.271a	18.606a	3.784a	3.891a	2.864a	10.539a
	DCB	15.339ab	7.611a	2.272a	77.900a	18.450a	3.732a	4.220a	3.337a	11.289a
	WW	15.278a	3.178a	1.061a	20.796a	14.136a	1.562a	2.068a	1.322a	4.953a
V. merrillii	DC	12.289b	2.600b	0.914b	18.971a	14.183a	1.594a	2.044a	1.176a	4.813a
	DCB	13.928ab	2.817ab	0.972ab	20.173a	14.150a	1.571a	2.058a	1.301a	4.930a

Values along the columns bearing same letters are not significantly different ($\alpha = 0.05$). WW denotes adequate watering (at 80 %FC), DC denotes dry down, DCB denotes dry down plus biochar. Shoot height (SH), number of leaves (LN), stem girth (SG), leaf area (LA), total leaf area (TLA), root length (RL), root fresh weight (RFW), stem fresh weight (SFW), leaf fresh weight (LFW), whole plant fresh weight (WPFW).

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Species	Trts	RSR	TLA (cm²)	LAR (cm ² g ⁻¹)	MDA ×10 ⁻³ (nM ml ⁻¹)	WUEt (g L-1 month ⁻¹	GPx ×10 ⁻⁴ (μM min ⁻¹ mg ⁻¹ FW)	CAT ×10 ⁻² μM min ⁻¹ mg ⁻¹ FW)	SOD×10 ⁻²⁽ units' min ⁻¹ mg ⁻¹ FW)
B. monandra	WW	0.174c	415.981a	17.858b	8.190b	1.026a	1.813a	4.934a	10.438a
	DC	0.304a	137.737c	22.334a	9.716a	0.731b	1.637a	3.813b	9.144a
	DCB	0.241b	299.799b	21.327ab	8.581b	0.844ab	1.734a	4.536ab	9.806a

	WW	0.294c	40.077a	25.676a	7.980a	0.223a	2.813a	4.496a	12.773a
D. lutescens	DC	0.398a	26.457b	22.942a	8.683a	0.209a	2.576a	3.904a	10.220a
	DCB	0.346b	33.757ab	25.650a	8.026a	0.215a	2.720a	4.150a	11.492a
	WW	0.242b	1140.137a	61.774a	6.363b	1.152a	3.680a	4.623a	13.499a
D. regia	DC	0.318a	432.284c	62.215a	8.223a	0.953a	3.092a	4.226a	11.475b
	DCB	0.302a	740.964b	67.813a	6.974b	0.971a	3.418a	4.396a	12.581ab
	WW	0.185b	765.379a	27.242a	6.024b	1.230a	4.277a	6.060a	15.583a
T. catappa	DC	0.268a	347.878b	29.721a	6.973a	0.982a	3.812a	4.433b	12.268b
	DCB	0.248a	607.742a	31.071a	6.182ab	1.043a	4.005a	5.713a	14.626a
	WW	0.350b	70.465a	28.188a	2.741a	0.276a	5.754a	6.845a	16.169a
V. merrillii	DC	0.380a	49.323a	27.074a	2.810a	0.263a	4.988a	4.773b	12.444b
	DCB	0.351b	58.896a	27.617a	2.778a	0.271a	5.299a	6.278a	13.729ab

Values along the columns bearing same letters are not significantly different (α = 0.05). Root shoot ratio (RSR), total leaf area (TLA), leaf area ratio (LAR), water use efficiency (WUEt), malondialdehyde content (MDA), guaiacol peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD).

The effect of dry down was profound on malondialdehyde (MDA) content of seedlings (Table 2). Dry down significantly (P<.05) increased MDA content by 18.6, 29.2 and 15.8% in B. monandra, D. regia and T. catappa seedlings respectively, relative to values under well watering and no such increase was found for other UFTS seedlings. Activities of antioxidant enzymes: superoxide dismutase (SOD), guaiacol peroxidase (GPx) and catalase (CAT) differed among the UFTS. The lowest activities were found for seedlings under dry down conditions (Table 2). Under dry down treatment, activities of SOD and CAT declined significantly (P<.05) in B. monandra seedlings (~ 21.3 and 26.8 % respectively) and in D. lutescens seedlings (30.6 and 41.7%). Superoxide dismutase activity significantly (P<.05) reduced by 15.0% in T. catappa seedlings while activity of CAT in D. regia seedlings significantly (P<.05) reduced by 28.9% compared with activities for well-watered seedlings. Under dry down also decreased the activities of SOD and GPx among seedlings. Biochar amendment of dry down condition appear to ameliorate the negative effects of high soil water deficit (dry down condition) such that antioxidant enzymes activities of SOD, CAT and GPx were statistically similar to seedlings under adequately watering (Table 2).

Generally, decreases in antioxidant enzyme ativities under un-amended dry down treatment follow the order: D. lutescens > B. monandra > D. regia > T. catappa > V. merrillii. Biochar amendment of dry down decreased SOD and CAT activities of seedlings in decreasing order of: D. lutescens > D. regia > T. catappa > B. monandra > V. merrillii and increase GPx activity in the order: B. monandra > D. lutescens > D. regia > T. catappa > V. merrillii. The chlorophyll contents of UFTS seedlings decreased under dry down condition. The contents of chlorophyll a, chlorophyll b and total chlorophyll were significantly lowered by \sim 46.3, \sim 49.4 and \sim 47.7% in B. monandra; 38.9, 41.6 and 40.1% in D. regia; 17.6, 27.3 and 22.0% in T. catappa, 32.7, 38.8 and 35.5% in D. lutescens; and 31.0, 33.3 and 32.1% in V. merrillii respectively (Table 3). The contents of light capturing pigments decreased significantly (P<.05) by 27.9, 32.5 and 30.0% respectively in D. lutescens and by 34.6, 39.3 %, 36.8% in D. regia and and 30 % in V. merrillii seedlings seedlings upon biochar amendment of dry down condition. Dry down treatment without biochar amendment decreased chlorophyll a concentration in the following order: D. regia > B. monandra > D. lutescens > V. merrillii > T. catappa while the trends of chlorophyll b content followed the order: D. regia > D. lutescens > B. monandra > V. merrillii > T. catappa (Table 3). Under dry down, decreasing Car/Chl ratio is: V. merrillii > B. monandra > D. regia > D. lutescens > D. regia > D. regia > D. regia > D. lutescens > D. regia > D. r

Dry down treatment without biochar amendment enhanced accumulation of proline, TSS, Asc and TPC (Table 3). Accumulation of proline, ASC and TPC significantly (P<.05) increased by ~ 34.1, 12.5 and 14.1% respectively in V. merrillii seedlings, TSS and ASC contents remarkably (P<.05) increased by ~ 19.0 and 22.7% for B. monandra seedlingorder of s while only ASC content increased considerably (P<.05) by \sim 17.4% in D. lutescens seedlings (Table 3). The highest increment of carotenoids 45.3% for D. regia (45.3%) under dry down treatment without biochar treatment while the lowest increment (3.9%) was detected in V. merrillii seedlings under biochar-dry down condition (Table 3). Relative to their contents under well watering, dry down condition increased the concentrations of proline, total soluble sugar (TSS), ascorbic acid (ASC) and total phenolic acid (TPC) in D. regia seedlings by about 30.8, 21.6, 13.5 and 30.4% while the contents of these biomolecules spiked by ~ 21.0, 14.1, 28.0 and 18.6% in T. catappa seedlings.

Species	TRT	Chl a (µM g-1FW)	Chl b (µM g-1FW)	TotChl (μM g-1FW)	Car (µM g-1FW)	Chl a/b	Car/Chl	Proline (μM g-1FW)	TSS mg/100mg	ASC (mM g-1FW)	TPC (mgGAE g-1FW)	RWC (%)
B. monan- dra	WW	11.711a	9.728a	21.440a	4.092a	1.189a	0.273b	7.846a	12.810b	18.824b	0.438a	67.793a
	DC	6.289b	4.927b	11.216b	4.685a	1.279a	0.598a	8.412a	15.249a	23.095a	0.547a	42.406b
	DCB	9.327ab	7.794ab	17.120ab	4.309a	1.156a	0.375b	8.292a	14.342ab	21.311ab	0.506a	49.636b
D. lute- scens	WW	12.633a	10.655a	23.289a	5.163a	1.236a	0.235b	8.849a	15.506a	20.831b	0.676a	71.437a
	DC	8.506b	6.519b	15.026b	6.094a	1.341a	0.509a	10.780a	17.166a	24.449a	0.758a	50.131b
	DCB	9.117b	7.198b	16.315b	5.864a	1.293a	0.442ab	10.279a	15.938a	22.235ab	0.721a	57.237b
D. regia	WW	14.457a	12.747a	27.204a	3.780a	1.141a	0.150b	12.540b	15.629b	22.857b	1.028b	68.536a
	DC	8.839b	7.444b	16.283b	5.493a	1.238a	0.421a	16.408a	19.009a	25.933a	1.341a	55.309b
	DCB	11.901a	10.371a	22.273a	4.430a	1.144a	0.248b	14.867ab	17.595a	24.716ab	1.187ab	56.623b
T. catappa	WW	13.518a	11.121a	24.639a	1.750a	1.223b	0.077a	13.372b	17.244b	32.063b	1.305b	81.171a
	DC	11.133b	8.080b	19.213b	1.745a	1.400a	0.096a	16.177a	19.682a	41.032a	1.548a	60.060b
	DCB	12.767ab	9.479ab	22.245ab	1.414a	1.362a	0.071a	14.750ab	18.835ab	35.756b	1.423ab	66.536b
V. merrillii	WW	14.442a	13.100b	27.542b	1.158a	1.111a	0.043a	14.666b	16.613a	34.242b	1.229b	84.416a
	DC	9.961b	8.743a	18.704a	1.571a	1.146a	0.111a	19.661a	19.000a	38.526a	1.402a	70.968b
	DCB	12.407ab	11.339b	23.746ab	1.276a	1.106a	0.066ab	17.611ab	17.202a	36.160ab	1.322ab	74.722b

Table 3: Effects of watering regime and biochar on biochemical constituents of seedlings of urban forestry tree species (UFTS).

Values along the columns bearing same letters are not significantly different ($\alpha = 0.05$). Relative water content (RWC), chlorophyll a content (Chl. A), chlorophyll b content (Chl. b), total chlorophyll content (TotChl), ratio of chlorophyll a to chlorophyll b (Chl a/b), ratio of carotenoids to total chlorophyll content (Car/Chl), total soluble sugar (TSS), ascorbic acid content (ASC.), total phenolic acid content (TPC).

Among UFTS, increment in TSS follows the trend of: B. monandra > D. regia > T. catappa > V. merrillii > D. lutescens. The increase in accumulated proline follows the order; V. merrillii > D. regia > D. lutescens > T. catappa > B. monandra, the increment in Asc content was of the oder: B. monandra > T. catappa > D. regia > D. lutescens > V. merrillii whereas increment in TPC was of the order: B. monandra > D. regia >T. catappa > V. merrillii > D. lutescens. However, biochar amendment of dry down treatment increased the accumulation of proline, TSS, ASC and TPC in the UFTS. Proline accumulation among UFTS follows the trend: V. merrillii > D. regia > T. catappa > D. lutescens > B. monandra while that of TSS was of the order; V. merrillii > B. monandra > D. regia > T. catappa > D. lutescens. Further, ASC content of biochar-amended dry down followed the order; T. catappa > B. monandra > D. lutescens > D. regia > V. merrillii whereas TPC was of the trend; D. regia > B. monandra > T. catappa > V. merrillii > D. lutescens (Table 3).

Drought susceptibility and aesthetic performance of UFTS

Drought susceptibility index (DSI) values were deployed to determine the effect of biochar and watering regime on shoot height of the UFTS seedlings (Table 4). Results indicated decreasing order of shoot height among the species as: D. regia > T. catappa > V. merrillii > B. monandra > D. lutescens. Effects of dry down on seedling leaf area in decreasing trend of: D. regia > D. lutescens > B. monandra > T. catappa > V. merrillii while DSI values of plant leaf area follows the order; B. monandra > D. regia > T. catappa > D. lutescens > V. merrillii. Biochar susceptibility index (BSI) showed biochar enhancement of plant leaf area in decreasing order of: D. regia > D. lutescens > B. monandra > V. merrillii > T. catappa. The increment in carotenoids contents in seedlings under dry down condition followed the trend V. merrillii > D. regia > T. catappa > D. lutescens > B. monandra. Biochar susceptibility index values showed decreasing order of: V. merrillii > D. lutescens > D. regia > T. catappa > B. monandra. Dry down significant (P<.05) enhanced root: shoot ratio by: ~ 74.7, 37.7, 53.1, 25.5 and 13.1% for Bauhinia, Delonix, Terminalia, Dypsis and Veitchia seedlings. Upon inclusion of biochar, root:shoot of B. monandra, D. regia, T.

catappa and D. lutescens remarkably (P<.05) increased by ~ 38.5, 30.1, 41.7 and 15.3% (Table 4). The ratio of carotenoids to total chlorophyll content (Car/Chl) of seedlings was high under dry down compared with well watering treatment.

 Table 4: Effects of watering regime and biochar on aesthetic performance of UFTS seedlings.

Species	TRT	RSR	RWC (%)	LAR (cm ² /g)	HGR	DQI (g)	WUEt (g L-1 month-1
B. monandra	WW	100c	100a	100b	100a	100a	100a
	DC	183.543a	63.740b	123.748a	103.117a	83.082c	71.512c
	DCB	142.073b	76.774ab	118.360a	97.481a	93.854b	81.985b
D. lutescens	WW	100c	100a	100a	100a	100a	100a
	DC	136.253a	71.078b	88.482b	102.782a	103.406a	98.688a
	DCB	118.176b	71.928b	98.208a	105.063a	97.835a	97.907a
D. regia	WW	100b	100a	100a	100a	100a	100a
	DC	137.965a	81.421b	103.884a	85.271b	108.522a	85.819b
	DCB	131.574a	82.330b	113.441a	92.837ab	103.045a	87.137b
T. catappa	WW	100b	100a	100b	100a	100b	100a
	DC	155.199a	74.417b	111.195ab	83.121b	111.562a	84.148b
	DCB	140.952a	82.182b	117.165a	90.790b	106.488ab	88.329b
V. merrillii	WW	100a	100a	100a	100a	100a	100a
	DC	117.468a	85.261b	108.159a	94.845a	105.194a	98.309a
	DCB	111.594a	89.016b	110.220a	100.543a	101.632a	101.130a

Values along the columns bearing same letters are not significantly different ($\alpha = 0.05$). Root shoot ratio (RSR), relative water content (RWC), leaf area ratio (LAR), ratio of shoot height to stem girth (HGR), Dickson quality index (DQI), water use efficiency (WUEt), guaiacol peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD).

Non biochar-amanded dry down significant (P<.05) decreased Car/Chlratios by ~ 119.0, 180.7, 116.6 and 122.0% for B. monandra, D. regia, D. lutescens and V. merrillii seedlings compared with biochar amendmentand well-watered seedlings. The DSI values of plant leaf area followed the order; B. monandra > D. regia > T. catappa > D. lutescens > V. merrillii. Under dry down condition, total plant biomass followed decreasing order of: B. monandra > T. catappa > D. regia > V. merrillii > D. lutescens. Following biochar amendment, drought susceptibility shows decreasing trends of total plant biomass among UFTS as: B. monandra > D. regia > T. catappa > V. merrillii > D. lutescens for WPDW and B. monandra > D. regia > T. catappa > V. merrillii > D. lutescens (Table 4). Increment in carotenoids contents of seedlings under dry down followed the trend V. merrillii > D. regia > T. catappa > D. lutescens > B. monandra. Drought susceptibility index showed reduction in relative water content of leaves among UFTS seedlings in both the non-amended and biochar amended dry down condition in the order of: V. merrillii < D. regia < T. catappa < B. monandra < D. lutescens.

Discussion

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Among the UFTS evaluated, differences were obtained in growth, physiology, and biochemical constituents under well

watering and dry down conditions with or without biochar ammendment. The significant decrease in leaf production by D. regia, T. catappa and especially B. monamdra seedlings could have resulted from deciduous habit of these species under high soil moisture deficit condition. This observation may constitute drought avoidance strategies in these species. The remarkable low number of fronds, shoot height and stem girth of V. merrillii and D. lutescens seedlings under extreme water deficit stress is similar to the reported findings on morphometrics of oil palm seedlings under the same condition [46,47]. Previous studies had observed a similar reduction in the leaf RWC of olive trees [48] under soil water deficit. The low chlorophyll contents of seedlings under dry down conditions is consistent with the findings of Silva-Pinheiro et al. (2016) on B. monandra and Soliman et al. (2015) on D. regia. Such decreases in chlorophyll contents could be related to elevated ROS formation induced by water deficit stress with consequences on photosynthesis and carbon assimilation [49,50]. The relatively high chlorophyll content in V. merrillii and D. regia seedlings under dry down condition could indicate species drought tolerance ability or inherently rich in chlorophyll pigment. The lack of significant impact of drought on carotenoids content of UFTS seedlings probably suggests other photosystem protective mechanisms other than carotenoids. These species might have sunscreen their photosynthetic apparatus by increasing osmolytes contents or enhanced activities of SOD, CAT and GPx or APx. Kesharvarz-Afshar et al. (2016) reported similar findings on Silybum marianum.

The differences observed in osmolyte concnetrations (proline, total soluble sugar, ascorbic acid, phenolic acid and total flavonoids) among seedlings indicate species-specificity of these traits. Changes in osmolytes concnetrations have been reported as a strategy to maintain osmotic potential (Aranjuelo et al., 2010) [23] and enhancing oxidative stress tolerance [51]. Generally, there was high ascorbic acid content (Asc) among the UFTS seedlings under dry down condition which may confirm that mesic UFTS seedlings enrouted the ascorbic acid peroxidase (APx) pathway to combat the drought-induced oxidative stress. Osmolytes, especially ascorbic acid supply electrons for the activation of antioxidant enzymes especially APx for scavenging ROS causing dismutation of H2O2 to water and molecular oxygen under water deficit stress [52, 53]. The significantly higher content of proline and total soluble sugar (TSS) in B. monandra seedlings and greater accumulation of proline, TSS and total phenolic acid (TPC) in T. catappa and D. regia seedlings under soil water deficit could indicate drought tolerance strategy in the species [25, 26]. The contents of proline, soluble sugar and phenolic acid appeared as plausible ecological markers for monitoring drought stress. This result agrees with the findings of [25,26,54] on D. regia. Proline and TPC contents were higher under dry down for V. merrillii seedlings compared with D. lutescens. Similar increase in the proline content had been reported in oil palm [55,56] and coconut palm [35]. [57,35] obtained low accumulation of proline in coconut under moisture stress for water balance regulation.

In this study, increases in contents of osmolytes were found in seedling leaves under biochar -amendment of dry down soil. This implies that as soil dries out, water is released from micro- and meso-pores of biochar amended soil resulting in low production of ROS and increased activities of enzymatic antioxidants. Biochar contains high contents of cations which may promote increased accumulation of osmotic active substances such as K+ in the plant tissues, resulting in an improved water uptake (Gaskin et al., 2010) and reduced quantity of ROS formation. The significant increase in MDA content of B. monandra, T. catappa and D. regia seedlings under dry down condition may be related to chloroplast membrane function. However, the dehydration of cytoplasm would have resulted in damage to photosynthetic apparatus due to higher levels of accumulated ROS. Under extreme water deficit stress condition, remarkable increase in the MDA content had also been observed in Eucalyptus globulus [58], Jathropha curcas [59]. In this study, V. merrillii and D. lutescens was least affected by lipid peroxidation due to low level of accumulated MDA under dry down condition. Thus, V. merrillii seedlings specifically exhibited relatively higher drought tolerance than semi deciduous species and such observation may be attributed to efficient regulation of stomata movement and enzymatic antioxidants activities. Plants

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increase the activities of antioxidant enzymes (CAT, SOD, APx and manage ROS accumulation under drought [60]. Increased activity of enzymatic antioxidants under stress conditions for protection from oxidative damage [61,62]. However, dry down treatment suppressed the activities of CAT and SOD in B. monandra and D. lutescens seedlings revealing higher susceptibility of species to oxidative stress.

Drought susceptibility of tested UFTS seedlings (using drought susceptibility indices)

Observations on the drought susceptibility (using drought susceptibility indices: DSI) showed that the UFTS seedlings differed in susceptibility to soil moisture deficit stress. Higher susceptibility of B. monandra than D. regia seedlings were consistent with the results of Hassanein (2015) on these species. The activities of SOD in T. catappa and CAT in D. regia declined significantly under dry down condition (more drought susceptible species) compared with V. merrillii seedlings which upregulated enzymatic activities (SOD, CAT and GPx) under moisture deficit. The UFTS evaluated tended to survive water deficit stress using avoidance mechanism by allocating more carbon resources to the root relative to shoots for enhancement of water extraction. In particular, B. monandra, D. regia, T. catappa and V. merrillii seedlings had reduced aboveground biomass compared with root biomass while D. lutescence seedlings tended to favour the shoot system compared with root in carbon allocation.

Contrary to the decrease in the chlorophyll a/b ratio reported for several agricultural crops [62,63]. The Chl a/b ratio increased significantly in T. catappa seedlings and slightly in other UFTS under water deficit which depicts the differential response of chlorophyll synthesis and degradation between agricultural crops and trees under drought. A similar increase in the Chl a/b of date palm was reported by Shareef et al. (2020). The biosynthesis/ degradation of photosynthetic pigments is relevant as plant stress tolerance mechanism underlying abiotic stress [64,65]. Leaf carotenoids content (LCar) is an important indicator of plant physiological status and are known to shield the photosynthetic apparatus against photo-oxidation by quenching ROS, particularly singlet oxygen under extreme condition of water stress (Caretto et al,. 2002), [66]. Under high soil moisture deficit stress, decreases in Car/Chl ratio follow the trend: Veitchia > Bauhinia > Delonix > Dypsis > Terminalia. This observation may indicate the susceptibility of chloroplast of UFTS seedlings to photo-oxidation under drought.

Biochar-enhancement of UFTS tolerance of soil moisture deficit

Biochar amendment of dry down treatment improved growth traits of T. catappa; and V. merrillii seedlings under dry down condition significantly. Such improvements could have stemmed from biochar improvement of soil properties [35, 67-69] and that drought mitigating capacity of biochar is species-specific [67,68]. Addition of biochar to dry down treatment could not effectively mitigate the negative impact of moisture stress as eflected in statistically lower leaf relative water content of UFTS seedlings under these treatments. This may indicate the existence of a threshold for biochar efficiency when adopted for drought alleviation in plants. Biochar amendment of dry down treatment maintained the levels of chlorophyll contents of B. monandra, D. regia, T. catappa and V. merrillii seedlings via reduction of droughtinduced oxidative stress and maintenance of metabolic processes and increased activity of anti-oxidant enzymes [42,70]. Previous studies reported positive influence of biochar on water stressed Eucalyptus globulus (Amaral, 2014), Phragmites karka [71] sand barley [60]. The results were ascribed to biochar-enhanced reduced oxidative stress, lipid peroxidation and enzymatic antioxidants. Our results establish the differentials response of seedlings to biochar application in particular under dry down condition for improved aesthetic values of UFTS. In addition to the drought mitigating potential of biochar is its relevance for conditioning urban soil for nursery production for improving growth and aesthetic value of UFTS [73-88].

Conclusion

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Treatment (well watering, dry down treatment and biochar amendment or not) effects was profound on the growth and physiological attributes, biochemical constituents and drought susceptibility of seedlings of urban forest species evaluated. Seedling attributes (root and shoot biomass, relative water content, plant leaf area, relative growth and net assimilation rates total leaf chlorophyll and carotenoids contents, ratios of chlorophyll to carotenoids) were significantly lower under dry down treatment relative to well watering. Dry down treatment elicited changes in growth and physiological attributes and biochemical constituents of seedlings of the evaluated UFTS. Dry down treatment enhanced the accumulation of osmolytes (proline, total soluble sugar (TSS), total flavonoids (TFC) and crude protein contents) and elicited changes in enzymatic activities (reduced Superoxide Dismutase Catalase activities and increased Guaiacol Peroxidase and Malondialdehyde Contents of seedlings.

Biochar amendment of dry down treatment enhanced values of growth, physiological and biochemical attributes of UFTS seedlings and modified enzymatic antioxidant activities. Biochar amendment appears to mitigate the negative effects of moisture deficit stress (dry down treatment) promoted accumulation of osmolyte (proline, TSS, ASC and TPC) and reduced enzymatic antioxidant activities (SOD, CAT and GPx. The strategies for improved drought tolerance exhibited by UFTS seedlings include improvement of antioxidant capacity, osmotic adjustment capacity (accumulation of osmolytes for protection of photosynthetic system) and reduced enzymatic antioxidant activities (SOD and CAT). The drought mitigating capacity of UFTS using sawdust biochar was affirmed. Study confirmed the drought susceptibility of the evaluated UFTS seedlings. Increasing order of drought tolerance of UFTS were B. monandra, D. regia, T. catappa, D. lutescens and V. merrillii.

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