

ECOPHYSIOLOGICAL RESPONSES OF LEAVES AND FINE ROOTS OF *INDOCALAMUS BARBATUS* MCCLURE SEEDLINGS TO HEAT AND DROUGHT

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Abstract

This study investigated the impact of global warming and the resulting hot and arid climate on the growth and afforestation of *Indocalamus barbatus* McClure seedlings. Different naturally occurring conditions of heat and drought were simulated in order to observe the ecophysiological responses to them in 2a *I. barbatus* seedlings, such as leaf gas exchange, fine root and leaf water potential, and antioxidase activity. The results showed that the following. 1) 2a *I. barbatus* seedlings responded differently to different heat and drought conditions; elevated temperatures significantly increased the transpiration rate and stomatal conductance, but non-stomatal limitations caused a decrease in the photosynthetic rate and restricted diurnal fluctuations in photosynthesis under extreme heat conditions. 2) Under moderate drought and its compounded conditions with heat, stomatal limitations caused a decreased photosynthetic rate. 3) Under severe drought and its compounded conditions with heat, the physiological processes of seedlings were severely impacted, and this was further compounded by the effects of high temperature, resulting in significantly decreased rates of transpiration, photosynthesis, stomatal conductance, and water use efficiency. 4) In the control, high temperature, and extreme temperature groups, water potential decreased more dramatically in fine roots than in mature leaves. 5) The effects of heat and drought on three types of antioxidases in the fine roots and mature leaves were consistent; superoxide dismutase activity in fine roots was higher than in mature leaves, while the reverse was observed for catalase and peroxidase activity. Therefore, fine roots are a critical structure in the adaptation of *I. barbatus* seedlings to heat and drought, as water absorption organs are more sensitive than transpiration organs. This study showed that *I. barbatus* seedlings are relatively resistant to heat and drought, and are able to increase the ecophysiological adaptability of the leaves and fine roots within certain temperature and humidity ranges. In particular, under compounded conditions, heat and drought have a synergistic effect on growth performance in plants. Extreme drought, however, results in damage to gas exchange mechanisms in leaves, as well as an impaired water intake in leaves and fine roots. Therefore, *I. barbatus* is not suitable for long-term cultivation under extreme drought condition, as high temperatures will aggravate the aforementioned damage.

Key words: Compounded conditions, *Indocalamus barbatus* McClure seedlings, Gas exchange, Water potential, Antioxidase.

Introduction

The atmospheric–oceanic–terrestrial interaction effects emerging as a result of global warming have caused a rise in the frequency and severity of low-probability and extreme climate events (White *et al.*, 2001; Lloret *et al.*, 2012; Zafar *et al.*, 2017). In China, extreme shifts in climate in areas along and south of the Yangtze River have manifested as increases in annual rainfall and the frequency of extreme precipitation events, while abnormally high pressures in subtropical zones of the Pacific Ocean have caused the increased occurrence and severity of natural disasters, such as droughts, floods, and extreme heat (Li *et al.*, 2008; Wang *et al.*, 2012). These sustained extreme heat and drought conditions pose a challenge to sustainable development of the forestry.

Experimental results obtained by Xu *et al.*, (2011) indicated that the compounded stress of heat and drought facilitated photosynthesis, transpiration, and stomatal conductance in *Robinia pseudoacacia* seedlings; however, there were no apparent increases in physiological indicators under either condition, suggesting that compounded heat and drought stress had different effects on different plant species. Beebe *et al.*, (2017) have suggested that increases in the severity and duration of drought stress result in increased plant litter, while heat slows developmental processes in plants, but that the two effects are not additive. At the whole-plant level, the

cooperative response of aboveground and underground organs to interactions between different environmental factors when faced with complex climate change is currently unclear (Carvalho *et al.*, 2015). Therefore, it is necessary to consider the collective temperature and moisture factors that affect plant growth, development, and functions if accurate predictions are to be made of the ecophysiological processes in plants under future climate conditions. A large number of domestic and foreign studies on the individual effects of heat or drought stress have been reported (Mittler, 2006; Wang *et al.*, 2012; Kursar *et al.*, 2009; Zafar *et al.*, 2017); however, studies on the compounded effects are relatively rare (Xu *et al.*, 2011; Carvalho *et al.*, 2015; Beebe *et al.*, 2017).

Indocalamus barbatus McClure, a dwarf bamboo species with large green leaves, has a relatively high reproductive ability and high landscaping value in China. At present, studies on the photosynthesis, transpiration, and stomatal characteristics of dwarf bamboo have been limited to single environmental factor. In the present study, a greenhouse cultivation experiment was conducted on *I. barbatus* seedlings under simulated heat and drought conditions. The fine roots and mature leaves of the seedlings were studied to investigate the relationships between relevant ecophysiological processes, and to analyze the seedlings' ability to resist and recover from heat and drought conditions.

Materials and Methods

Overview of experimental site and experimental materials: The experimental site used in the study is a grapevine nursery in Changxing County, Zhejiang Province (119°45'51" E, 30°58'59" N). The site has a subtropical maritime monsoon climate, with an annual average temperature of $15.6 \pm 0.5^\circ\text{C}$, effective accumulated temperature of $5,750^\circ\text{C}$, frost-free period of 239 d, annual average rainfall of 1,309 mm, annual average sunshine duration of 1,810.3 h, and annual average percentage of sunshine of 41%. The soil on the site mainly consists of red soil developed in acidic magma and sedimentary rock. The soil layer within the experimental site is relatively thick, reaching 1.5 m in places. The organic matter content in woodland soil (w) is 30.2 g/kg on average, with w (total nitrogen; TN) of $1.16\text{--}2.24 \text{ g}\cdot\text{kg}^{-1}$, w (available phosphorus) of $98.00\text{--}101.76 \text{ mg}\cdot\text{kg}^{-1}$, and w (available potassium) of $54.75\text{--}92.61 \text{ mg}\cdot\text{kg}^{-1}$.

In October 2015, in a large greenhouse constructed from white plastic film, healthy disease- and pest-free 1a *I. barbatus* seedlings were selected for the experiment. Seedlings measuring 40–50 cm, each pruned to one branch with 3–5 leaves and with roots measuring 5–10 cm, were transplanted into $3.0 \text{ m} \times 1.2 \text{ m} \times 0.20 \text{ m}$ (length \times width \times height) seedbeds (external soil was used to construct a 30 cm tall protective wall to ensure breathability). Rows of seedlings were spaced 20–30 cm apart; after planting, an infiltration system was used for irrigation (Cui *et al.*, 2004), using rows of PVC pipes spaced 10–15 cm apart and laid flat on the seedbed. Under a pressure head of 10–40 cm, the infiltration rate was $22.09 \pm 4.77 \text{ cm}^3\cdot\text{m}^{-1}\cdot\text{min}^{-1}$. Soil was irrigated to the point of saturation, after which the soil surface was covered with a 2–3 cm thick layer of river sand and a 1–2 cm thick layer of humus to retain moisture in the seedbed. During the experimental period, the site was routinely monitored and irrigated. The space from ground level to 40 cm above ground level in the greenhouse was not covered by plastic film in order to facilitate ventilation. The roof of the greenhouse was sealed with film, which was opened on sunny days and closed during rainy periods. The baseline soil nutrients were as follows: organic matter, $20.08 \pm 3.61 \text{ g}\cdot\text{kg}^{-1}$; w (TN), $2.64 \pm 0.55 \text{ g}\cdot\text{kg}^{-1}$; w (available phosphorus), $15.29 \pm 3.88 \text{ mg}\cdot\text{kg}^{-1}$; and w (available potassium), $40.50 \pm 8.37 \text{ mg}\cdot\text{kg}^{-1}$. The soil pH was 5.1 ± 0.3 .

Experimental design: Simulations of water loss and recovery under heat and drought conditions were initiated in March 2016. 2a *I. barbatus* seedlings were divided into control, heat, and extreme heat groups, and subjected to nine types of treatment in total (Table 1). Heat stress was induced as follows: across the seedbeds of all heat and extreme heat groups, silicon carbon heating tubes (black tubes, 1.8 cm in diameter and 1.0 m long, with four connected tubes making up one row and rows spaced 0.5 m apart, creating a three-row formation) were installed 1.6 m above ground level. A thermostat was suspended in a central position 1.0 m from the ground for digital temperature control (Zhang *et al.*, 2013). The controlled

day and night temperatures across the different treatment groups are shown in Table 1. The soil water potential for the different treatments was as follows: normal, heat and extreme heat treatments, -30 to 0 kPa (soil humidity: $37.86\% \pm 5.95\%$); moderate drought and its compounded treatments, -30 to -60 kPa (soil humidity: $27.95\% \pm 2.64\%$); and severe drought and its compounded treatments, -60 to -90 kPa (soil humidity: $18.25\% \pm 1.73\%$). Soil water potential was monitored and data were collected using a soil moisture meter (with a built-in sensor). The criterion for severe drought and its compounded treatments was designated as a lack of wilting in the experimental seedlings at noon every day.

Table 1. Experimental design and environmental temperatures.

Groups	Treatments	Temperatures (°C)
Control	Normal	Day 12~28 Night 7~20
	Moderate drought	
	Severe drought	
Heat	Heat	Day 33~35 Night 30~33
	Heat + Moderate drought	
	Heat + Severe drought	
Extreme heat	Extreme heat	Day 38~40 Night 35~38
	Extreme heat + Moderate drought	
	Extreme heat + Severe drought	

Measurement of transpiration rate, stomatal conductance, and net photosynthetic rate: Fifty-eight days after initiating the simulation experiment and before entering the high temperature summer, the third fully extended mature leaf (counted from the top) on the longest branch of each germinated seedling was selected from each treatment group. Transpiration rate (T_r), net photosynthetic rate (P_n), and stomatal conductance (G_s) were measured hourly between 09:00–15:00 on a sunny day using the Li6400 portable photosynthesis system (LI-COR, USA). This was repeated across 5 plants from each treatment.

Measurement of water potential in leaves and fine roots: At the same time as measuring gas exchange parameters in leaves, other seedlings were selected from the same treatment and water potential was measured in fine roots ($\leq 2 \text{ mm}$) and mature leaves at 2 h intervals between 06:00–20:00. A WP4-T Dewpoint Potentiometer (Decagon, USA) and a pressure chamber were used to measure the water potential in mature leaves and fine roots, respectively. Measurements were repeated across 5 plants for each treatment.

Measurement of antioxidase activity: Samples were obtained immediately after conclusion of the experiment. Leaves were selected from the middle portion of seedlings to test for antioxidase activity. Sampling was repeated 3 times in order to obtain average values. Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activity was measured. For each sample, 0.2 g of the leaf was washed and placed in a mortar, to which was added 1.6 mL of $50 \text{ mmol}\cdot\text{L}^{-1}$ prechilled phosphate buffer (pH 7.8). The mixture was ground into a homogenate in an ice bath, transferred into a centrifuge tube, and centrifuged at

4°C for 20 min at 12,000 r·min⁻¹. The resulting supernatant was then extracted for enzyme activity testing; SOD, CAT, and POD activity was measured using the nitro blue tetrazolium method, ultraviolet absorption method, and a guaiacol colorimetric assay, respectively (Hao *et al.*, 2013).

Data processing: Experimental data were processed using a one-way ANOVA and least significant difference (LSD) test were used to compare the significance of differences between treatments.

Results and analysis

Transpiration rate, net photosynthetic rate, stomatal conductance, and water use efficiency in leaves: Tr, Pn, and Gs all showed decreasing trends of normal > moderate drought > severe drought, heat > heat + moderate drought > heat + severe drought, and extreme heat > extreme heat + moderate drought > extreme heat + severe drought, suggesting that all three parameters significantly impacted soil humidity across the various groups (Fig. 1). Single peaks were observed for Tr, Pn, and Gs under the compounded heat + severe drought and extreme heat + drought conditions; single peaks were also observed for values of Gs under severe drought conditions alone.

Compared to severe drought condition, the compounded heat + severe drought and extreme heat + severe drought conditions resulted in a significant decrease in the Tr peak values ($p < 0.05$), whereas double

peaks were observed for all other treatments at approximately 10:00 and 14:00, suggesting that transpiration was significantly hindered under these two conditions. Peak Tr values under the heat condition differed significantly from the normal condition ($p < 0.05$), indicating that heat could facilitate transpiration in seedlings, given an adequate water supply in soil. Under extreme heat condition, the average peak values were higher than those observed in the normal condition.

Compared to the control group, peak values of Pn in the extreme heat group showed a corresponding significant decrease ($p < 0.05$), compared to a non-significant decrease in the heat group ($p > 0.05$). The peak Pn values in the severe drought and extreme heat + severe drought conditions were significantly different ($p < 0.05$), suggesting that the responses of *I. barbatus* seedlings to heat were not apparent. The peak Pn values for the moderate drought, extreme heat + moderate drought, heat + moderate drought, and extreme heat + moderate drought conditions were significantly different ($p < 0.05$). This suggests that, under extreme heat condition, Pn in *I. barbatus* seedlings responded significantly to temperature. Peak Pn values did not respond significantly to temperature under the heat + moderate drought condition. Within the daily progression, photosynthetic rates in seedlings under the severe drought, heat + severe drought, and extreme heat + severe drought conditions were essentially zero by 15:00, which suggests that severe drought is a key factor influencing photosynthesis in *I. barbatus* seedlings.

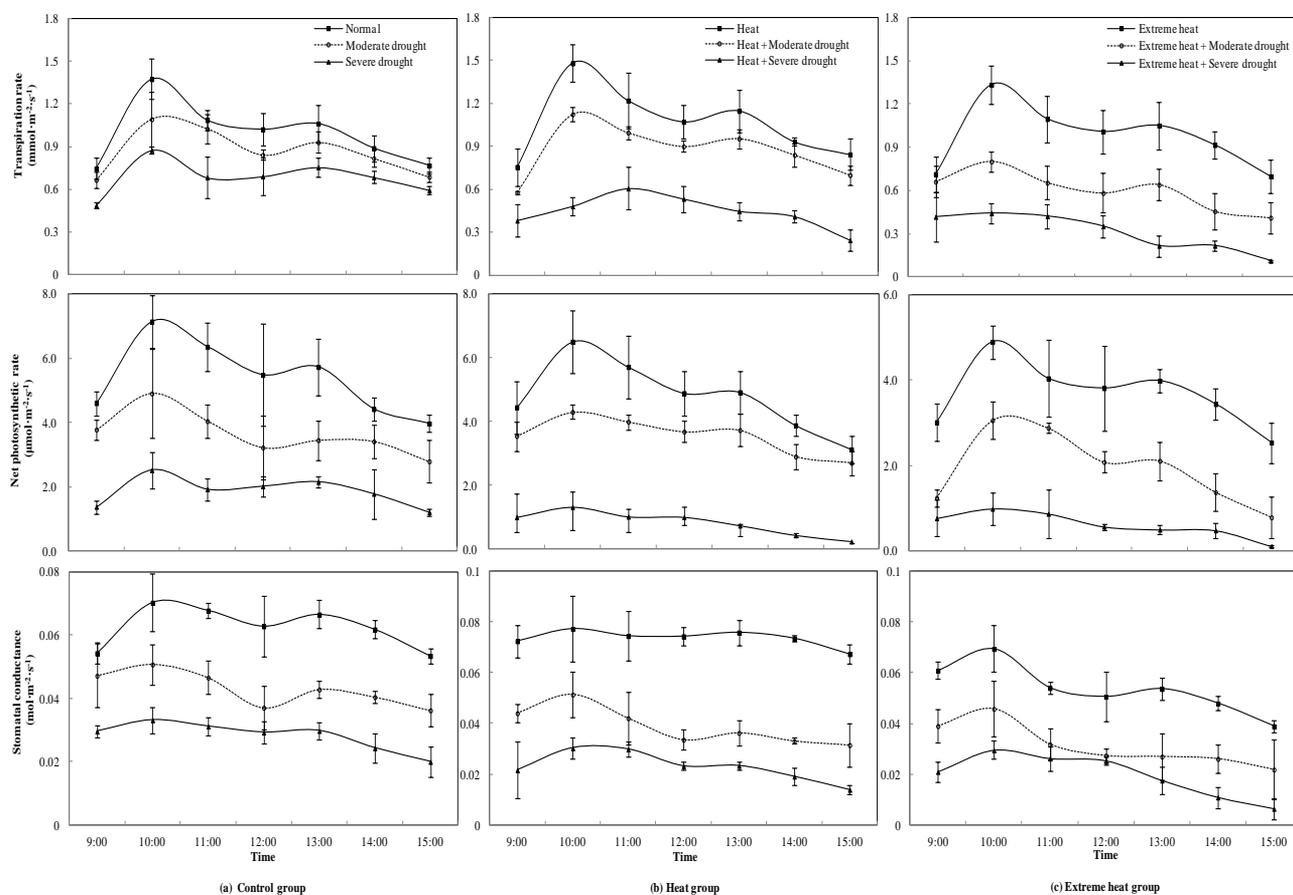


Fig. 1. Diurnal processes of Tr, Pn, and Gs of *I. barbatus*'s under different treatments.

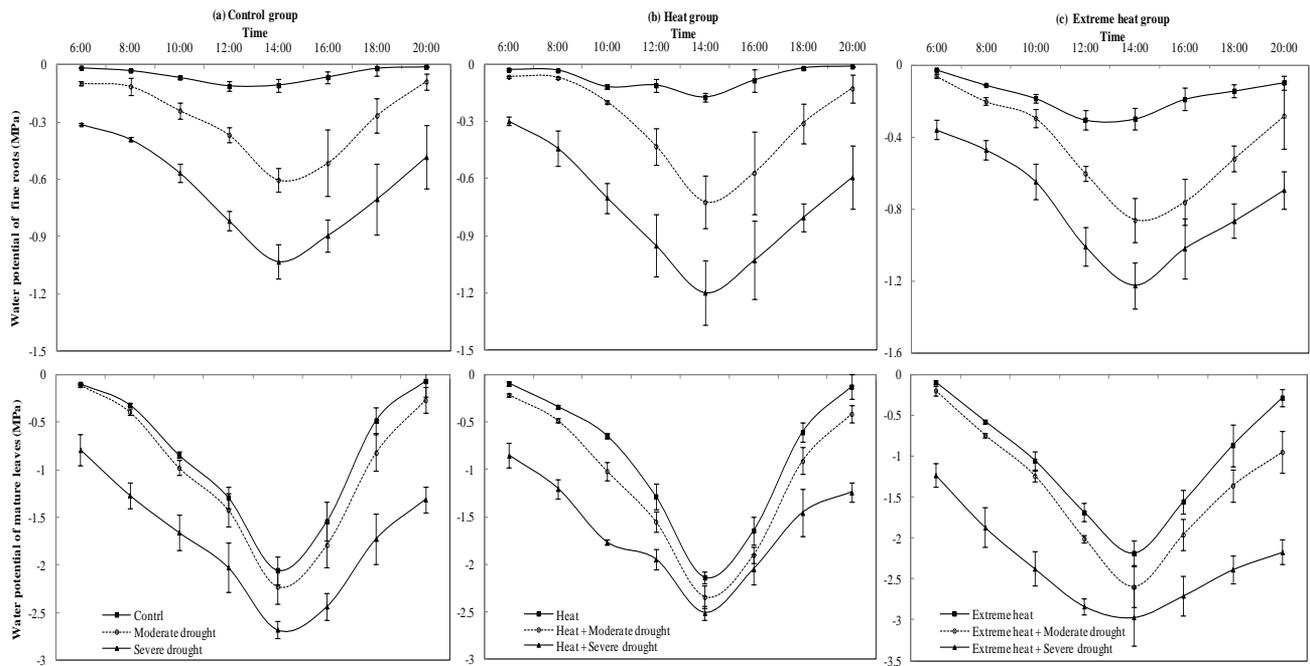


Fig. 2. Diurnal varieties of water potential of *I. barbatus*'s fine roots and mature leaves under different treatments.

Compared to the control group, peak G_s values for the heat and extreme heat groups did not differ significantly ($p > 0.05$). In addition, the average values obtained at each time point within the daily progression showed increases under heat condition, in direct contrast with those obtained under extreme heat condition. This suggests that *I. barbatus* seedlings open their stomata in response to high temperatures, and close in response to extreme temperatures, but not to an extent that affect their physiological processes. Compared to the normal, heat, and extreme heat conditions, G_s values under the severe drought, heat + severe drought, and extreme heat + severe drought conditions decreased drastically under drought conditions, which is consistent with the results obtained for T_r and P_n .

Dynamic changes in water potential in fine roots and mature leaves: Daily fluctuations of water potential under transpiration pull appeared as valleys of different depths (Fig. 2). Water potential was relatively high under the normal, heat, and extreme heat conditions, and remained high in fine roots throughout the day, with no major changes. Water potential started to decrease at 08:00, reaching a maximum valley depth at 14:00. Under the normal and heat conditions, water loss was essentially recovered after noon, with no valley observed between 08:00–18:00. The results for the extreme heat condition differed significantly from the other two conditions from 08:00–18:00 ($p < 0.05$), indicating that the fine roots of seedlings increased T_r and decreased water potential in response to extreme heat condition; as drought conditions intensified across treatments, the water potential in fine roots also decreased, yielding significant results ($P < 0.05$). Deeper valleys were observed as the extent of drought increased, and the recovery process following water loss at noon was significantly delayed. Daily changes in average water potential were not apparent in the normal

and heat conditions compared to other conditions, and water losses were essentially recovered by approximately 16:00. Daily changes in average water potential in the fine roots of seedlings subject to the moderate drought, heat + moderate drought, and extreme heat + moderate drought conditions can be interpreted as follows: differences between the moderate drought and extreme heat + moderate drought conditions were significant ($p < 0.05$), with the recovery observed at 20:00 only in moderate drought condition being visible at 06:00, indicating that transpiration was enhanced as temperature increased. Although the water potential in the three aforementioned conditions showed significant responses to temperature, there were no significant differences ($p > 0.05$) in the severe drought, heat + severe drought, and extreme heat + severe drought conditions; water loss from fine roots was not recovered by 20:00 in any of these three treatments, indicating that severe drought had already disrupted normal physiological progress in these seedlings.

The water potential in mature leaves of *I. barbatus* seedlings was maintained at relatively high dynamic levels in soil with a relatively high water potential, and that water loss from leaves could be recovered effectively; a low water potential in soil caused low-level fluctuations in water potential in leaves, which could not be rapidly recovered (Fig. 2). Among the three conditions of control group, daily variations in leaf water potential were similar to those for conditions of heat group. Differences between normal and moderate drought conditions were not significant ($p > 0.05$), but differed significantly from daily fluctuations in the severe drought condition ($p < 0.05$). Water potential fluctuations in leaves from the heat and extreme heat groups followed a similar pattern; extreme heat + severe drought condition showed the greatest values of maximum valley depth among all conditions. Within the control group, daily fluctuations under the moderate

drought and normal conditions were similar, but different from those under severe drought condition; in the normal and moderate drought conditions, fluctuations mainly manifested as a dramatic decrease around noon, a shorter valley period, and smaller decreases around 06:00–08:00, when atmospheric evaporation was relatively weak. A sustained, rapid decrease was observed from 09:00–14:00, when evaporation and transpiration rates increased drastically, with the lowest values occurring in the afternoon. The subsequent recovery led to the formation of narrow and pointed valleys. In severe drought condition, a rapid decrease was observed from 06:00–8:00, followed by sustained minimum values between 10:00–14:00, which then slowly increased to form a wide, shallow valley. The extent of the decrease at noon was smaller, however; the valley stage showed an apparent early onset, and recovery was delayed. On the whole, the aforementioned results suggested that as available water resources decreased in the soil substrate, dynamic water potential levels in the mature leaves of *I. barbatus* seedlings were also decreased, extending the valley period and delaying the dehydration period in leaves. This pattern was similar in the heat and extreme heat groups, but was especially prominent in the extreme heat + severe drought condition; the water potential was lowest in these leaves when transpiration forces were strongest (from 10:00–14:00), when there was a significantly lower water potential in the mature leaves than under the extreme heat and extreme heat + moderate drought conditions. Dramatic decreases in water potential in the period around noon under moderate drought and its respective compounded conditions, as well as severe drought and its respective compounded conditions, suggested that the maintenance mechanisms of water potential did not provide an adequate response in conditions of dehydrated soil. Normal, heat, and extreme heat conditions exerted the strongest suppressive effects during the periods of strongest transpiration (10:00–16:00), during which the water potential in mature leaves showed sustained low levels. This suggests that seedling leaves were capable of activating intrinsic water flow regulation mechanisms to stop the sustained decrease in water potential, serving a critical protective function for their photosynthetic machinery and other intracellular structures.

Antioxidase activity in fine roots and mature leaves: In the control group, SOD activity in fine roots was highest in moderate drought condition, but this did not differ significantly from the normal condition (Table 2, $p > 0.05$). SOD activity significantly decreased with increasing drought severity ($p < 0.05$), indicating damage to the SOD system under extreme drought condition. This was consistent with patterns in SOD activity observed in the heat and extreme heat groups. SOD activity levels in mature leaves were similar to those in fine roots. Average CAT activity in fine roots of the control group increased under moderate drought conditions, but decreased significantly in severe drought condition ($p < 0.05$). Patterns in CAT activity under heat group were similar to those in the control group, where an initial rise was

followed by a decrease; this indicated that, under normal or elevated temperatures, *I. barbatus* seedlings experienced inhibited physiological functions under severe drought conditions. CAT activity in fine roots decreased significantly under the extreme heat + moderate drought and extreme heat + severe drought conditions ($p < 0.05$), indicating that it was significantly impacted by heat. In both the control and heat groups, patterns in POD activity in fine roots and mature leaves were similar to those observed for CAT activity, demonstrating an initial increase in the fine roots, followed by a decrease. Overall, the average activity observed for these three antioxidases indicated that the heat, extreme heat, moderate drought, and heat + moderate drought conditions had a positive impact through enhancing resilience in fine roots and mature leaves, while the average values for antioxidase activity observed in seedlings under severe drought and its compounded conditions were indicative of inhibitory effects caused by these treatments.

Discussion

The impact of heat on transpiration, photosynthesis, and stomatal conductance in *I. barbatus* seedlings: Plants demonstrate the highest Pn under optimal temperature and soil humidity conditions (Taylor *et al.*, 2012). The present study showed that severe drought and its compounded conditions heavily impacted normal physiological processes in *I. barbatus* seedlings. High and extreme temperatures both compounded these effects, responding with a several-fold or several dozen-fold reduction in Tr, Pn, and Gs. High temperatures increased transpiration significantly, resulting in a slight increase in Gs; extreme heat, however, led to a significantly decreased Pn, and narrowed the range within which Pn varied throughout the day; in contrast, there were no decreases in either Gs or Tr, which instead demonstrated average increases. This may well be due to the abundant supply of water under the heat and extreme heat conditions. This is consistent with the results obtained by Ranney (1994) in a study of *Betula platyphylla* leaves subjected to leaf temperatures of 25–40°C under abundant water supply conditions. Under conditions involving drought or its compounded effects, Pn, Tr, and Gs simultaneously decreased, but Pn showed a higher level of decrease than Tr, indicating that decreased water efficiency hindered the supply of CO₂ in chloroplasts, due to stomatal limitation factors (An *et al.*, 2010; Zhu *et al.*, 2016). Therefore, adequate temperature increases in the domestication and cultivation of heat-tolerant species may suffice in altering their gas exchange processes (Taylor *et al.*, 2012; Zhu *et al.*, 2016). The present study showed that Tr, Pn, and Gs in *I. barbatus* seedlings responded differently to different heat and drought conditions, and that the seedlings demonstrated a satisfactory resistance to extreme temperatures. Plant survival was not threatened by extreme heat + moderate drought, but the species was unsuited for long-term cultivation in arid environments. Heat, moderate drought, and heat + moderate drought conditions were however conducive to the domestication and cultivation of *I. barbatus* seedlings.

Table 2. Antioxidant enzyme system of *I. barbatus*'s fine roots and mature leaves under different treatments.

Organ	Activity of protective oxidase systems	Control group			Heat group			Extreme heat group		
		Normal treatment	Moderate drought	Severe drought	Heat	Heat + Moderate drought	Heat + Severe drought	Extreme heat	Extreme heat + Moderate drought	Extreme heat + Severe drought
Fine roots	SOD (U·g ⁻¹ ·min ⁻¹)	460.43±37.29a	494.33±17.25a	299.67±13.66c	453.97±41.23ab	515.07±59.64a	312.38±85.15bc	486.77±52.90a	518.40±27.92a	296.43±36.96c
	CAT (U·kg ⁻¹ ·min ⁻¹)	882.40±164.51a	1030.31±250.74a	493.23±204.67b	956.53±244.77a	1131.42±210.06a	417.17±162.84b	1164.25±206.00a	835.13±88.14a	358.47±219.18b
	POD (U·mg ⁻¹ ·min ⁻¹)	23.56±3.88ab	26.83±3.29a	20.71±1.38b	26.85±1.25a	27.32±2.57a	21.52±2.48b	30.26±2.03a	28.83±3.84a	14.94±2.56c
Mature leaves	SOD (U·g ⁻¹ ·min ⁻¹)	352.11±27.54bc	415.80±45.65ab	329.24±33.62cd	397.90±28.32b	421.83±44.93ab	347.23±32.74bcd	451.15±13.55a	443.43±19.57a	305.96±17.60d
	CAT (U·kg ⁻¹ ·min ⁻¹)	1232.38±258.60ab	1453.28±186.69ab	868.16±120.58c	1212.20±118.38b	1164.07±171.25b	759.53±230.80c	1493.03±174.73a	1323.11±164.85ab	768.71±93.12c
	POD (U·mg ⁻¹ ·min ⁻¹)	36.44±3.14b	38.16±4.09ab	29.37±3.11c	36.12±3.10b	37.79±4.02ab	28.21±5.22c	41.64±2.54a	38.53±2.94ab	26.63±1.53c

The data in the table are average value ± standard deviation

Different letters indicate significant differences at the 0.05 probability level

Effects of heat on water potential in leaves and fine roots of *I. barbatus* seedlings: Different plant organs have different or even contradictory adaptation strategies towards environmental change (Roessner *et al.*, 2006). In a study conducted on *Olea europaea*, Dichio *et al.*, (2006) found that a combined action of the root system and leaves was needed to sustain photosynthesis under drought stress conditions, and water potential in plant root systems and leaves is affected by the combined effects of soil water level and temperature (Kang *et al.*, 1999). The present study showed that, apart from the physiological damage observed in heat + severe drought and extreme heat + severe drought conditions, a low Tr at noon was also observed in seedlings subject to other conditions, but fluctuations in the water potential in fine roots and mature leaves were not observed. Water potential fluctuations throughout the day manifested as a single valley, possibly due to the intrinsic properties of water exchange, transport, and water heat capacity in plants, whereby the processes of water absorption in roots and transpiration were not completely synchronized, resulting in a phenomenon in which absorption lagged behind transpiration (Memmi *et al.*, 2015). The water potential in fine roots and mature leaves began to recover at approximately 14:00, which was consistent with results reported by Cui *et al.*, (2004), indicating that a change in water absorption in *I. barbatus* seedling tissues occurred at this time; under the control, moderate drought, heat, heat + moderate drought, and extreme heat conditions, water potential in fine roots and mature leaves only recovered at 20:00. As the severity of drought increased, the water potential in fine roots and mature leaves decreased. An increase in heat stress intensity also increased the cost needed for the recovery of normal water potential in fine roots and mature leaves. At the same time, the water potential in soil similarly affected both the daily ranges of valley values and the progression of water potential recovery in fine roots and mature leaves, but had different effects on their respective daily fluctuations. Higher soil water potential caused small fluctuations in the peak water potential values in both leaves and roots, compared to lower soil water potential, which caused small and large fluctuations in the valley values of water potential in leaves and roots, respectively. This trend was most apparent between 10:00–16:00, especially under severe drought and its compounded conditions (i.e., in dehydrated soil where capillary action was disrupted); the decrease in water potential in fine roots was more significant than that in the leaves. Therefore, in terms of water potential, the indirect responses of the absorption organs were more sensitive to elevated atmospheric evaporation than the direct responses of the transpiration organs, suggesting that water uptake in fine roots was affected by other inhibitory forces, or was in a state of deficiency (Kursar *et al.*, 2009; Blessing *et al.*, 2015).

Effects of heat and drought on protective oxidase systems in *I. barbatus* seedlings: In the present study, it was found that, as temperature increased, seedlings increased their intrinsic SOD, POD, and CAT activity in

order to eliminate active oxygen and avoid heat damage, thereby avoiding damage to their enzyme defense systems even under extreme heat conditions. Under moderate drought and its compounded conditions, SOD, POD, and CAT activity did not decrease, suggesting that the effects of heat and extreme heat on soil moisture and transpiration pull were insufficient to cause serious damage to the enzymatic defense systems of plants. However, severe drought and its compounded conditions resulted in decreased activity of all three enzymes, as well as a decrease in the photosynthetic ability of leaves; this suggested that, under these conditions, active oxygen species exceeded the regulatory abilities of protective antioxidases. This demonstrates that *I. barbatus* seedlings have a relatively high resistance to temperature and drought, and that their intrinsic antioxidase activity allows them to survive in moderate drought, heat, or extreme heat conditions. Within a given range, heat and drought stimulate an increase in antioxidase activity, and oxidation damage to organ tissues is reversible; under severe drought conditions. However, active oxygen species cause severe damage to cellular membrane systems, and these effects are compounded by heat.

To a large extent, the activity in the fine roots of a plant determines its survival and resistance. An *et al.*, (2010) report that SOD, CAT, and POD activity in *Periploca sepium* seedlings demonstrated different change trends in immature leaves, aging leaves, and roots, and that the protective enzyme response was most sensitive in fine roots. The present study showed that, while heat and drought had relatively consistent effects on SOD, POD, and CAT activity levels in *I. barbatus* seedlings, these levels differed significantly between fine roots and mature leaves three antioxidases. In particular, SOD activity was significantly elevated in fine roots compared to mature leaves, while the reverse was observed for CAT and POD activity. SOD activity constitutes a critical factor in the protective system; a higher degree of activity in fine roots than leaves proves that the former have an elevated ability in mediating the metabolism of active oxygen species and eliminating active oxygen species (Hao *et al.*, 2013), but that the latter are superior in terms of mediating physiological and biochemical metabolism and development. This suggests that fine roots are essential organs in the adaptation of *I. barbatus* seedlings to heat and drought, and that physiological integration and cooperation between different organs enables adaptation to warm environments with alternating humidity levels. Therefore, in unfavorable environments, *I. barbatus* seedlings tend to prioritize organs that protect the vitality of their own survival in order to improve their ability to survive in adverse conditions (Carvalho *et al.*, 2015), and different tissues and organs have different response mechanisms to heat and drought stress.

Conclusions

a) Under conditions where water was abundant in the soil, *I. barbatus* seedlings showed increases in Tr and Gs in response to heat. Non-stomatal limitations that the raise of leaf temperature, the decrease of chloroplast and

Rubisco activity were the main causes of decreased photosynthesis under extreme heat conditions, while stomatal limitations were the main cause of decreased photosynthesis under drought and its respective compounded conditions.

b) Daily fluctuations in water potential in fine roots and mature leaves showed that, under soil conditions where capillary action was disrupted, indirect responses by water uptake organs to atmospheric evaporation were more sensitive than direct responses by transpiration organs in leaves.

c) Heat and extreme heat did not seriously affect the protective antioxidase systems of the plants. Within a given range, heat and drought increased the enzymatic activity of antioxidases; fine roots were more adept at metabolizing active oxygen species than mature leaves, showing that they served as an important organ in the species' adaptation to heat stress.

d) Ecophysiological parameters, indicators of water potential in organs, and measurements of antioxidase activity indicate that *I. barbatus* seedlings are relatively resistant to heat and drought.

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