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EFFECTS OF HYDROGEN PEROXIDE ON SEED GERMINATION, SEEDLING GROWTH AND PHYSIOLOGICAL CHARACTERISTICS OF *BOMBAX CEIBA* AFTER HEAT SHOCK

YANLING ZHENG[1], XINHUA YIN[2] AND HUANCHENG MA[1*]

[1]Key Laboratory of State Forestry Administration on Biodiversity Conservation in Southwest China, Southwest Forestry University, Kunming, 650224, China
[2]Department of Plant Sciences, The University of Tennessee, Jackson, Tennessee 38301, USA
[*]Corresponding author's email: flashingzyl@163.com

Abstract

Bombax ceiba can grow in the dry-hot valley of southwestern China where adult trees produce many seeds, but few seedlings are found around the mature trees. Previous studies have shown that high temperature is a limiting factor for seed germination of *B. ceiba*. The effects of seed pretreatment with hydrogen peroxide (H_2O_2) on seed germination, seedling growth and physiological characteristics in seedlings were studied after seeds were heat shocked. After heat shock, germination percentage decreased for seeds without H_2O_2 treatment (untreated seeds) compared to the seeds that experienced no heat shock (control). Photosynthetic activity was decreased in the seedlings of untreated seeds, which was partially caused by the enhanced heat dissipation. Soluble sugar and proline did not play a role in osmoprotectance as they did not accumulate. Pretreatment of seeds with 80 mM of H_2O_2 improved seed germination rate and increased photosynthetic efficiency of seedlings relative to the no treatment on seeds, which might be related with the H_2O_2-induced activity of superoxide dismutase. However, pretreatment of seeds with a higher concentration of H_2O_2 at 120 mM accelerated chlorophyll degradation and photoinhibition in seedlings. In conclusion, seeds of *B. ceiba* can be primed with 80 mM of H_2O_2 to improve seedling survival under high temperature stress.

Key words: Abiotic stress, Antioxidant system, Compatible solutes, Reactive oxygen species.

Introduction

The balance between light absorption and light utilization for photosynthetic assimilation is liable to be broken under various stresses (Flexas & Medrano, 2002; Asada, 2006), and the excessive absorbed energy could lead to oxidative stress by overproducing reactive oxygen species (ROS) (Foyer & Harbinson, 1994). Oxidative stress could disrupt specific molecules which affects metabolism or even leads to plant death (Utriainen & Holopainen, 2001; Asada, 2006). Membranes have long been identified as the sites of response to various environmental stresses (Levitt, 1980). Malondialdehyde (MDA) is a product of lipid peroxidation, and is often used to indicate the degree of oxidative damage (Zhang *et al*., 2012). Thylakoid membranes of chloroplasts are primary sensors of environmental changes and the photochemical process of photosynthesis is therefore liable to be affected by stresses (Krause & Weis, 1991; Anderson *et al*., 1997). Chlorophyll fluorescence can be used to evaluate the photosynthetic performance and stress tolerance of plants (Maxwell & Johnson, 2000), and the maximum quantum yield of photosystem II (PSII) (Fv/Fm) is a good indicator of photoinhibition (Andersson & Barber, 1996).

In nature, plants have developed various mechanisms to avoid or tolerate different stresses (Chaves *et al*., 2003). Although the excessive absorbed energy can cause photodamage to photosynthetic apparatus, plants can avoid such damage by dissipation of excitation energy as heat (Müller *et al*., 2001). In addition, under adverse conditions, plants can change the pigment content to decrease the absorption of light (Zarco-Tejada *et al*., 2000). The formation of ROS can be enhanced under stresses, but the antioxidant system (enzymatic and non-enzymatic) can prevent overproduction of ROS (Mittler, 2002). The non-enzymatic system includes lipophilic compounds such as carotenoids. The antioxidant enzymatic system contains several enzymes, including peroxidase (POD) which regulates both ROS production and scavenging, superoxide dismutase (SOD) which dismutates O_2^- to hydrogen peroxide (H_2O_2), and catalase (CAT) which catalyses the conversion of H_2O_2 to water and molecular oxygen (Passardi *et al*., 2005; Mai *et al*., 2010). Some stresses such as extreme temperatures, drought, and salinity can lead to osmotic stress of plants. However, plants can accumulate compatible solutes including soluble carbohydrates and proline to maintain water uptake (Silva *et al*., 2010).

Despite their damage potential, ROS also serve as signaling molecules in the responses of plants to stresses (Gomes & Garcia, 2013; Suzuki, 2015). For example, H_2O_2 plays a key role in the signal transduction process which activates defense responses to various stresses (Liu *et al*., 2010; wang *et al*., 2014). The content of H_2O_2 has been shown to increase in response to stresses such as heat, drought and salinity in a variety range of plant species (Dat *et al*., 1998; Talbi *et al*., 2015; Lu *et al*., 2016). Investigations have also showed that exogenous application of H_2O_2 increases the tolerance of some plants to biotic and abiotic stresses. For instance, Wang *et al*. (2014) reported that exogenous application of H_2O_2 enhanced the thermotolerance of tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*). Liu *et al*. (2010) found that exogenous H_2O_2 improved tolerance of two cucumber ecotypes to osmotic stress. Hu *et al*. (2009) observed that H_2O_2 pretreatment mitigated cadmium stress in rice seedlings. In most cases, the improvement of stress tolerance induced by H_2O_2 pretreatment is partly due to the elevated antioxidant status (Hu *et al*., 2009; Wang *et al*., 2014).

Bombax ceiba is a tall tree buttressed at the base, widely found throughout India and other parts of the tropical and sub-tropical Asia, Australia, and Africa (Verma *et al*., 2011; Chaudhary & Khadabadi, 2012). *Bombax ceica* is a multipurpose tree species providing food, fodder, and fibre (Jain *et al*., 2011), and almost all plant components are of medicinal importance (Chaudhary & Khadabadi, 2012). *Bombax ceiba* can grow up to more than 20 m in height in the dry-hot valley but its natural regeneration by seedlings is difficult. Ballabha *et al*. (2013) also reported poor regeneration of *B. ceiba* in India. Seed germination and seedling establishment are considered the most vulnerable stages of plants to environmental stresses (Leck *et al*., 2008; Kolb & Barsch, 2010). Our previous study has demonstrated that high temperature is a limiting factor of seed germination in *B. ceiba* (Zheng *et al*., 2013). To improve the survival rate of forestation in the dry-hot valley of southwestern China, heat tolerance of *B. ceiba* during seed germination and seedling establishment should be enhanced. Seed priming is a simple and effective approach to enhance plant tolerance to various stresses (Bhanuprakash & Yogeesha, 2016). However, there is no related research on seed priming-induced heat tolerance of *B. ceiba*. This study was conducted to evaluate the effect of seed treatment with H_2O_2 on the tolerance of *B. ceiba* to heat stress during the early stages of seed germination and seedling establishment.

Material and Methods

Seed collection: Dry fruits (capsules) of *B. ceiba* containing mature seeds were collected from Lujiangba, Baoshan, Yunnan province (lat. 24° 54′ N, long. 98° 53′ E) in April 2014. The capsules were randomly harvested from 15 trees, and brought back to the laboratory within 5 d. The seeds were selected from the capsules manually and used immediately for the experiment.

Seed treatments with H_2O_2 and heat shock: Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2, respectively, for 24 h at 30°C before heat test. Four replicates of 25 seeds per treatment were randomly sampled, placed on top of two-layers of qualitative filter papers and moistened with distilled water in Petri dishes (10 cm diameter). Seeds were then heat shocked at 45°C for 2 h before seed germination test. Seeds that were not subjected to H_2O_2 pretreatment and heat shock were as the controls.

Seed germination test: After heat shock, the Petri dishes were then placed in a germination chamber at constant temperature of 30°C under a 12-h light photoperiod. The germination was conducted following the procedure adopted by Zheng *et al*. (2013). At the end of germination, fresh weight (SFW) and physiological characteristics of seedlings were determined. Germination percentage (GP), germination index (GI), and vigor index (VI) were determined according to Zheng *et al*. (2013).

Leaf pigments: Chlorophyll and carotenoid concentrations were determined according to Wang (2006). Absorbance of the extract were measured at 665 nm, 649 nm, and 470 nm wave lengths with a spectrophotometer (uv-2450).

Chlorophyll fluorescence: Three seedlings were selected from each treatment, and one leaf was sampled from each seedling. Fluorescence parameters were measured at indoor temperature with a *chlorophyll* fluorometer (PAM-2500, Walz, Germany). Seedlings were adapted in the dark for 30 min before measurements were conducted. In this paper, Fv/Fm, effective quantum yield of PS II (Y(II)), photochemical quenching coefficient (qP), non-photochemical quenching coefficient (NPQ), non-regulated (Y(NO)) and regulated (Y(NPQ)) non-photochemical energy loss in PS II as well as electron transport rate (ETR) were measured.

MDA content: MDA content was measured as described by Wang (2006). Absorbance was read at 450, 532, and 600 nm using a spectrophotometer (uv-2450, Shimadzu, Japan).

Proline content: Proline content was measured as described by Wang (2006). Absorbance was read at 520 nm with a spectrophotometer (uv-2450).

Soluble sugar: Soluble sugar content was measured as described by Wang (2006). The light absorption of the samples was estimated at 625 nm using a spectrophotometer (uv-2450).

Antioxidant enzyme activity: POD, SOD and CAT activities were measured as described by Wang (2006). For determination of POD, SOD and CAT activity, the absorbance of the supernatant was read at 470 nm, 560 nm and 240 nm, respectively.

Statistical analysis: The experimental design was completely randomized, with four replicates for seed germination and three replicates for leaf pigment concentrations, contents of MDA, proline, and soluble sugar, antioxidant enzyme activity, and chlorophyll fluoresence parameters. Statistical analyses were performed using SPSS 15.0 (IBM Corp., Armonk, NY). Seed germination percentage was subjected to arcsine transformation before statistical analysis. The data were subjected to one-way analysis of variance (ANOVA). Significant differences between treatment means were tested by Fisher's least significant difference (LSD) test at the 0.05 probability level.

Results

Seed germination and seedling growth: The embryonic roots of some seeds began to protrude from seed coats after 24 h soaking in water and H_2O_2. Seed GP decreased by 18.1% but GI, SFW, and VI did not change for untreated seeds (without pretreatment with H_2O_2) after heat shock compared to the unstressed control (Table 1). Compared to those of untreated seeds, GP of seeds pretreated with different concentrations of H_2O_2 did not change and SFW of seeds pretreated with 80 mM of H_2O_2 increased by 13.8%, although not significant. GI and VI of seeds pretreated with 40 and 80 mM of H_2O_2, respectively, increased over those of untreated seeds.

Table 1. Effects of hydrogen peroxide (H_2O_2) seed treatment on seed germination and seedling growth of *B. ceiba* after heat shock.

H_2O_2 (mM)	GP (%)	GI	SFW (g)	VI
Control	94.0 ± 7.7a	10.50 ± 0.69c	0.27 ± 0.04b	2.88 ± 0.60b
0	77.0 ± 6.8b	10.12 ± 0.71c	0.29 ± 0.03ab	2.98 ± 0.45b
40	79.0 ± 2.0b	16.08 ± 0.34a	0.31 ± 0.04ab	4.98 ± 0.73a
80	71.0 ± 8.9b	14.55 ± 2.31ab	0.33 ± 0.02a	4.77 ± 0.52a
120	70.0 ± 7.7b	12.96 ± 1.38b	0.25 ± 0.03b	3.23 ± 0.64b

GP: germination percentage, GI: germination index, SFW: seedling fresh weight, VI: vigor index
Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2 for 24 h, respectively, before heat test. For the heat test, seeds were heat shocked at 45°C for 2 h before seed germination test. Results represent treatment means of four replicates ± SD. The means followed by different lowercase letters within a column are significantly different by Fisher's least significant difference at $p \leq 0.05$

Table 2. Effects of hydrogen peroxide (H_2O_2) seed treatment on pigment composition of *B. ceiba* after heat shock.

H_2O_2 (mM)	a (mg/g)	b (mg/g)	a+b (mg/g)	a/b	Carotenoid (mg/g)
Control	12.71 ± 0.84a	4.60 ± 0.43a	17.30 ± 1.24a	2.77 ± 0.13b	2.10 ± 0.07a
0	11.38 ± 1.07a	3.58 ± 0.42b	14.96 ± 1.43a	3.19 ± 0.23a	2.01 ± 0.19a
40	12.81 ± 1.50a	4.20 ± 0.37ab	17.01 ± 1.87a	3.05 ± 0.08a	2.22 ± 0.29a
80	11.84 ± 1.63a	3.88 ± 0.45ab	15.72 ± 2.08a	3.05 ± 0.07a	2.07 ± 0.29a
120	8.64 ± 1.09b	2.78 ± 0.34c	11.43 ± 1.43b	3.11 ± 0.02a	1.64 ± 0.19a

Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2 for 24 h, respectively, before heat test. For the heat test, seeds were heat shocked at 45°C for 2 h before seed germination test. Results represent treatment means of three replicates ± SD. The means followed by different lowercase letters within a column are significantly different by Fisher's least significant difference at $p \leq 0.05$

Pigment composition: After heat shock, chlorophyll a, chlorophyll a+b, and carotenoid did not change but chlorophyll b was decreased and chlorophyll a/b was increased for seedlings from untreated seeds compared to those of the unstressed control (Table 2). Chlorophyll a/b and carotenoid of seedlings from seeds pretreated with different concentrations of H_2O_2 did not differ from those of seedlings from untreated seeds. Compared to those of the seedlings without seed treatment, chlorophyll a, chlorophyll b, and chlorophyll a+b did not differ for seedlings from seeds pretreated with 40 and 80 mM of H_2O_2 but decreased by 24.1%, 22.2%, and 23.6% respectively, for seedlings from seeds pretreated with 120 mM of H_2O_2.

Chlorophyll fluorescence: The Fv/Fm, Y(II), qP, and ETR was decreased, and Y(NPQ) and NPQ increased for seedlings from untreated seeds after heat shock compared to the unstressed control (Table 3). The Fv/Fm, Y(II), qP, and ETR increased firstly for seedlings from seeds pretreated with 40-80 mM of H_2O_2, and then decreased for seedlings from seeds pretreated with 120 mM of H_2O_2 compared to the seedlings without seed treatment. Y(II), qP, and ETR of seedlings from seeds pretreated with 80 mM of H_2O_2 increased over those of the seedlings from untreated seeds, but did not differ from those of the unstressed control. The NPQ of seedlings from seeds pretreated with 40 and 120 mM of H_2O_2 increased compared to that of the seedlings without seed treatment and the unstressed control. The Y(NPQ) of seedlings from seeds pretreated with 40 and 120 mM of H_2O_2 increased compared to that of the control. However, only Y(NPQ) of seedlings from seeds pretreated with 120 mM of H_2O_2 increased relative to that of the seedlings without seed treatment. Both NPQ and Y(NPQ) of seedlings from seeds pretreated with 80 mM of H_2O_2 decreased over those of the seedlings without seed treatment, but did not differ from those of the unstressed control. The Y(NO) did not vary among the seedlings with and without seed treatments and the unstressed control.

MDA content: After heat shock, MDA of seedlings from untreated seeds did not differ from that of the unstressed control, and it did not differ between the seedlings from treated and untreated seeds (Fig. 1). However, MDA of seedlings from seeds pretreated with 80 and 120 mM of H_2O_2 decreased relative to that of the control.

Antioxidant enzyme activity: The POD activity did not vary among the seedlings with and without seed treatments after heat shock and the unstressed control (Table 4). The SOD activity of seedlings from untreated seeds decreased by 47.2% compared to that of the control, and its activity of seedlings from seeds pretreated with 80 mM of H_2O_2 increased by 76.9% relative to that of the seedlings without seed treatment. The CAT activity increased in seedlings from both treated and untreated seeds compared to that of the control. The CAT activity of seedlings with 80 mM H_2O_2 seed treatment was the highest, although it did not differ significantly from that of the seedlings without seed treatment.

Soluble sugar and proline content: After heat shock, soluble sugar content of seedlings without seed treatment decreased compared to that of the control, and it did not vary among the seedlings with and without seed treatments (Fig. 2a). Proline content of seedlings from untreated seeds did not differ from that of the control (Fig. 2b). Proline content of seedlings with seed treatment increased with the increase of H_2O_2 concentration relative to that of seedlings from untreated seeds, but only the proline content of seedlings with 120 mM of H_2O_2 seed treatment increased significantly compared to that of the seedlings without seed treatment.

Table 3. Effects of hydrogen peroxide (H_2O_2) seed treatment on chlorophyll fluorescence characteristics of *B. ceiba* after heat shock.

H_2O_2 (mM)	Fv/Fm	NPQ	qP	Y(II)	Y(NPQ)	Y(NO)	ETR
Control	0.76 ± 0.01a	0.42 ± 0.08d	0.73 ± 0.03a	0.50 ± 0.03a	0.14 ± 0.02c	0.35 ± 0.03a	52.66 ± 3.45a
0	0.72 ± 0.03b	0.50 ± 0.20c	0.51 ± 0.01c	0.33 ± 0.20b	0.22 ± 0.10b	0.45 ± 0.12a	33.43 ± 1.29b
40	0.75 ± 0.02ab	0.61 ± 0.23b	0.59 ± 0.03b	0.38 ± 0.01ab	0.23 ± 0.06b	0.39 ± 0.06a	38.63 ± 0.93b
80	0.74 ± 0.01ab	0.41 ± 0.13d	0.78 ± 0.03a	0.53 ± 0.01a	0.14 ± 0.03c	0.34 ± 0.03a	53.40 ± 1.42a
120	0.71 ±0.01b	0.71 ± 0.13a	0.36 ± 0.06d	0.21 ± 0.12b	0.32 ± 0.04a	0.47 ± 0.10a	21.17 ± 12.55c

Fv/Fm: maximum quantum yield of photosystem II, NPQ: non-photochemical quenching coefficient, qP: photochemical quenching coefficient, Y(II): effective quantum yield of photosystem II, Y(NPQ): regulated non-photochemical energy loss in photosystem II, Y(NO): non-regulated non-photochemical energy loss in photosystem II, ETR: electron transport rate

Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2 for 24 h, respectively, before heat test. For the heat test, seeds were heat shocked at 45°C for 2 h before seed germination test. Results represent treatment means of three replicates ± SD. The means followed by different lowercase letters within a column are significantly different by Fisher's least significant difference at $p \leq 0.05$

Table 4. Effects of hydrogen peroxide (H_2O_2) seed treatment on activities of antioxidant enzymes of *B. ceiba* after heat shock.

H_2O_2 (mM)	SOD (U/g)	POD (U/min/mg)	CAT (U/min/g)
Control	172.31 ± 17.61a	14.25 ± 5.55a	17.09 ± 0.42c
0	91.00 ± 7.16b	14.99 ± 2.28a	32.88 ± 5.42ab
40	118.81 ± 18.71b	13.79 ± 2.56a	32.66 ± 2.75ab
80	161.02 ± 21.83a	17.50 ± 7.58a	39.35 ± 4.31a
120	113.09 ± 8.94b	18.84 ± 4.23a	31.96 ± 4.15b

SOD: Superoxide dismutase, POD: Peroxidase, CAT: Catalase

Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2 for 24 h, respectively, before heat test. For the heat test, seeds were heat shocked at 45°C for 2 h before seed germination test. Results represent treatment means of three replicates ± SD. The means followed by different lowercase letters within a column are significantly different by Fisher's least significant difference at $p \leq 0.05$

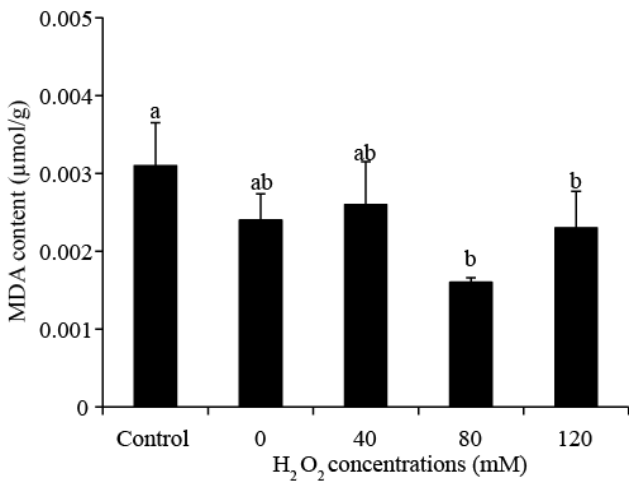

Fig. 1. Effects of hydrogen peroxide (H_2O_2) seed treatment on malondialdehyde (MDA) content of *B. ceiba* after heat shock. Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2 for 24 h, respectively, before heat test. For the heat test, seeds were heat shocked at 45°C for 2 h before seed germination test. Data points represent treatment means of three replicates ± SD. The bars indicated by different lowercase letters are significantly different by Fisher's least significant difference at $p \leq 0.05$.

Discussion

Effects of H_2O_2 seed treatment on the response of seed germination to heat shock: Seed germination is the most critical phase in plant life cycle due to its high vulnerability to various stresses (Rajjou *et al*., 2012). Our previous research showed that high temperature was one of the factors affecting seed germination of *B. ceiba* (Zheng *et al*., 2013). The results of this study also showed that GP of untreated seeds decreased when they were subjected to heat shock. As GI, SFW, and VI did not change, it indicated that germination rate and early seedling growth were not affected by the heat stress.

H_2O_2 is a major ROS which play improtant roles in endosperm weakening, mobilization of seed reserves, transmitting environmental cues and some other processes during seed germination (Gomes & Garcia, 2013). Compared to those of the control and untreated seeds, GI and VI of seeds pretreated with 40 and 80 mM of H_2O_2, respectively, increased after heat shock. Our results suggest that pretreatment of seeds with 40 to 80 mM of H_2O_2 increases germination rate of *B. ceiba* after heat shock. However, would H_2O_2 have similar effects in non-heat stressed seeds of *B. ceiba* is not clear. Some other studies also showed that exogenous application of H_2O_2 stimulated seed germination and curtailed mean germination time (Narimanow & Korystov, 1997; Wahid *et al*., 2007). The faster germination induced by seed priming might be due to enhanced pre-germination metabolic activities (Soon *et al*., 2000).

Effects of H_2O_2 seed treatment on the response of *B. ceiba* seedlings to heat shock: Besides seed germination, the metabolic processes of seedlings arised from primed seeds are affected by seed priming under various stresses (Jisha *et al*., 2013; Bhanuprakash & Yogeesha, 2016).

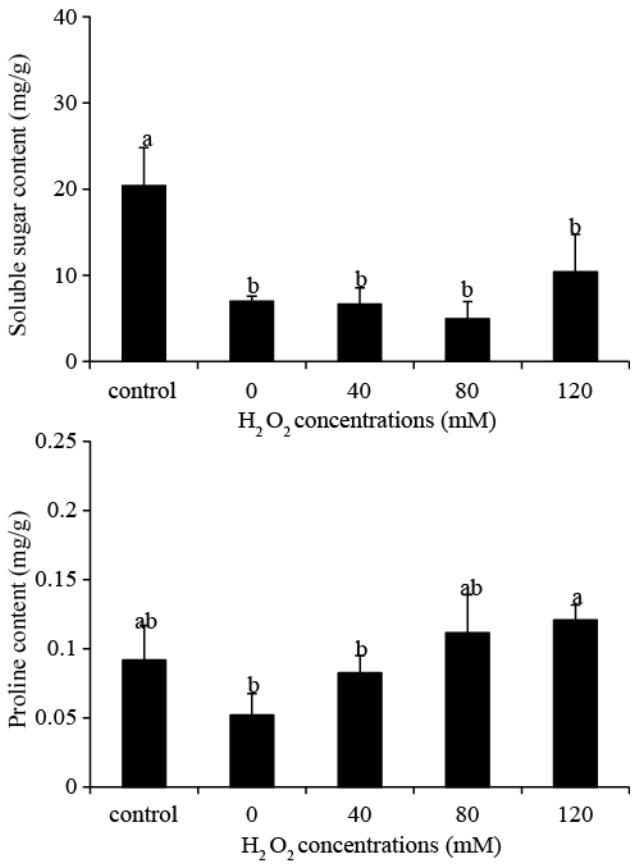

Fig. 2. Effects of hydrogen peroxide (H_2O_2) seed treatment on soluble sugar (A) and proline (B) contents of *B. ceiba* after heat shock. Seeds were soaked in 0, 40, 80, and 120 mM H_2O_2 for 24 h, respectively, before heat test. For the heat test, seeds were heat shocked at 45°C for 2 h before seed germination test. Data points represent treatment means of three replicates ± SD. The bars indicated by different lowercase letters are significantly different by Fisher's least significant difference at $p \leq 0.05$.

It has been reported that chlorophyll a/b can vary greatly depending on the physiological status of the plants, increasing under some types of stresses but decreasing under the other stresses (Pearcy & Sims, 1994; Schoefs *et al*., 1998; Kouril *et al*., 1999; Utriainen & Holopainen, 2001). For *B. ceiba*, increase in chlorophyll a/b for seedlings without H_2O_2 seed treatment after heat shock indicated that chlorophyll a might be less susceptible to degradation than chlorophyll b. Compared to the seedlings without H_2O_2 seed treatment, pigment content and composition of seedlings from seeds pretreated with low concentrations of H_2O_2 did not change after heat shock, but chlorophyll content of seedlings from seeds pretreated with 120 mM of H_2O_2 decreased. Our results suggest that the effect of H_2O_2 on pigment content is related to its concentration, and pretreatment of seeds with high concentration of H_2O_2 induces oxidative stress to seedlings of *B. ceiba* and promotes chlorophyll degradation. Similarly, Gechev *et al*. (2002) reported that the role of H_2O_2 was dependent on the dose of H_2O_2 applied, and high concentrations of H_2O_2 caused oxidative stress on tobacco.

Compared to those of the control, Fv/Fm, Y(II), qP, and ETR decreased but Y(NPQ) and NPQ increased for the seedlings without H_2O_2 seed treatment after heat shock. Our results suggest that photoinhibition occurs and the photoinhibitory processes are, at least partially, due to the enhanced heat dissipation which plays a photoprotective role. Many investigations have also suggested that photoinhibition is not due to damage, but because of increases in thermal dissipation (Demmig-Adams *et al*., 1996; Logan *et al*., 1997). The Fv/Fm, Y(II), qP, and ETR increased firstly for seedlings from seeds pretreated with 40-80 mM of H_2O_2 and then decreased for seedlings with 120 mM of H_2O_2 seed treatment compared to those of the seedlings without seed treatment. It is obvious that the role of H_2O_2 is concentration-dependent and a proper concentration of H_2O_2 can promote heat tolerance of *B. ceiba* at some extent. The Y(NPQ) and NPQ increased for seedlings with 40 and 120 mM of H_2O_2 seed treatments but did not change for seedlings from seeds pretreated with 80 mM of H_2O_2 compared to those of the control. This trend might be due to the fact that seedlings with 40 and 120 mM of H_2O_2 seed treatments were heat stressed and could avoid further damage through thermal dissipation; however, seedlings from seeds pretreated with 80 mM of H_2O_2 did not suffer from heat stress or just suffered less. These results were coincident with other fluorescence parameters including Y(II), qP, and ETR.

Although MDA is often used as an oxidative biomarker, some studies have shown that there is not a linear relationship between MDA content and stress intensity and change of MDA content might be dependent on stress type, stress intensity and plant tissues (Shi & Bao, 2007; Li, 2009). In the present study, MDA content of seedlings without seed treatment did not differ from that of the control after heat shock, and pretreatment of seeds with H_2O_2 did not have effect on MDA content. However, correlation analyses showed that MDA content did not correlate with chlorophyll content and chlorophyll fluorescence parameters (data not shown). Therefore, MDA content might not indicate the true redox state. Many studies have showed the positive role for antioxidant enzymes in stress tolerance. This study showed that POD activity did not change, SOD activity decreased, and CAT activity increased for seedlings without seed treatment after heat shock compared to those of the control. This observation indicated that CAT played a significant role in scavenging ROS after heat shock. Other studies have also suggested that different antioxidant enzymes of plants might have different response patterns when exposed to stresses (Mai *et al*., 2010; Weng *et al*., 2015; Amiri *et al*., 2016). Enzymatic patterns might be related to the adaptive mechanisms of plants to stresses. Recently, several studies have showed the stimulation of antioxidant systems in plants when exposed to exogenous H_2O_2 treatment (Gechev *et al*., 2002; Li *et al*., 2011). The SOD activities of seedlings from seeds pretreated with 80 mM of H_2O_2 increased compared to that of the seedlings without seed treatment, and the CAT activities of seedlings with 80 mM of H_2O_2 seed treatment were the highest among the seedlings with and without seed treatments. It is obvious that pretreatment of seeds with 80 mM of H_2O_2 is conducive to protect seedlings of *B. ceiba* against oxidative stress induced by heat shock. Similar results were reported that H_2O_2 could protect plants from oxidative stress by inducing a set of antioxidant enzymes (Gechev *et al*., 2002; Wang *et al*., 2014).

It has been reported that the osmotic substances can maintain cell turgor, prevent enzyme inactivation, and reestablish the celluar redox balance of stressed plants (Mahajan & Tuteja, 2005; Chaves *et al*., 2009; Krasensky & Jonak, 2012). Investigations showed that soluble sugar content increased for some plants but decreased for other plants after heat treatment (Gill *et al*., 2003; Liu *et al*., 2008; Gomathi *et al*., 2013; Yang *et al*., 2015). For *B. ceiba*, soluble sugar content of seedlings with and without seed treatments decreased after heat shock compared to that of the unstressed control. The different results among studies might be due to the fact that the role of soluble sugar in heat tolerance is related to plant species and/or other factors**.** It has been documented that accumulation of proline increases the tolerance of some plants to abiotic stress, and H_2O_2 at low concentrations can induce accumulation of proline (Guzel & Terzi, 2013). This study showed that only the proline content of seedlings from seeds pretreated with 120 mM of H_2O_2 increased compared to that of seedlings without seed treatment. Correlation analyses showed that proline content had no correlations with seedling growth, chlorophyll content, or chlorophyll fluorescence parameters (data not shown). Therefore, proline content was not related to the heat tolerance of *B. ceiba*. Kolodyazhnaya *et al*. (2009) reported that the correlation results between proline content and tolerance to abiotic stresses are rather inconsistent. This discrepancy may be due to the fact that different plants adopt different protective mechanisms under various stresses.

Conclusions

This study showed that heat shock decreased seed germination percentage but did not affect seed germination rate or seedling growth of *B. ceiba*. Pretreatment of seeds with 80 mM of H_2O_2 increased heat tolerance of *B. ceiba* during seed germination and early seedling growth. However, a higher concentration of H_2O_2 at 120 mM induced chlorophyll degradation and photoinhibition. In application, seeds of *B. ceiba* can be primed with an appropriate concentration of H_2O_2 to improve seedling survival under high temperature stress. As this study was focused on effects of H_2O_2 on seed germination and seedling characteristics after heat shock, how did seed priming with H_2O_2 affect seed germination and seedling growth of *B. ceiba* were not clear and should be explored in the following work. Additionally, drought and heat often occur simultaneously in the field; therefore, research about the effects of H_2O_2 seed treatment on tolerance of *B. ceiba* to the combined drought and heat stress is warranted for forestation in the dry-hot valley of southwestern China.

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