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

YANLING ZHENG¹, XINHUA YIN² AND HUANCHENG MA*∗

¹Key Laboratory of State Forestry Administration on Biodiversity Conservation in Southwest China, Southwest Forestry University, Kunming, 650224, China
²Department 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 (H₂O₂) 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 H₂O₂ 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 osmoprotection as they did not accumulate. Pretreatment of seeds with 80 mM of H₂O₂ improved seed germination rate and increased photosynthetic efficiency of seedlings relative to the no treatment on seeds, which might be related with the H₂O₂-induced activity of superoxide dismutase. However, pretreatment of seeds with a higher concentration of H₂O₂ at 120 mM accelerated chlorophyll degradation and photoinhibition in seedlings. In conclusion, seeds of B. ceiba can be primed with 80 mM of H₂O₂ 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 O₂ to hydrogen peroxide (H₂O₂), and catalase (CAT) which catalyses the conversion of H₂O₂ 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, H₂O₂ 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 H₂O₂ 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 H₂O₂ increases the tolerance of some plants to biotic and abiotic stresses. For instance, Wang et al. (2014) reported that exogenous application of H₂O₂ enhanced the thermostolerance of tall fescue (Festuca arundinacea) and perennial ryegrass (Lolium perenne). Liu et al. (2010) found that exogenous H₂O₂ improved tolerance of two cucumber ecotypes to osmotic stress. Hu et al. (2009) observed that H₂O₂ pretreatment mitigated cadmium stress in rice seedlings. In most cases, the improvement of stress tolerance induced by H₂O₂ 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 ceiba 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$_2$O$_2$ 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$_2$O$_2$ and heat shock:** Seeds were soaked in 0, 40, 80, and 120 mM H$_2$O$_2$, 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$_2$O$_2$ 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 fluorescence parameters. Statistical analyses were performed using SPSS 15.0 (IBM Corp., Armonk, NY). Seed germination percentage was subjected to arc sine 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$_2$O$_2$. Seed GP decreased by 18.1% but GI, SFW, and VI did not change for untreated seeds (without pretreatment with H$_2$O$_2$) 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$_2$O$_2$ did not change and SFW of seeds pretreated with 80 mM of H$_2$O$_2$ increased by 13.8%, although not significant. GI and VI of seeds pretreated with 40 and 80 mM of H$_2$O$_2$, respectively, increased over those of untreated seeds.
Table 1. Effects of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) seed treatment on seed germination and seedling growth of B. ceiba after heat shock.

<table>
<thead>
<tr>
<th>H\textsubscript{2}O\textsubscript{2} (mM)</th>
<th>GP (%)</th>
<th>GI</th>
<th>SFW (g)</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.0 ± 7.7a</td>
<td>10.50 ± 0.69c</td>
<td>0.27 ± 0.04b</td>
<td>2.88 ± 0.60b</td>
</tr>
<tr>
<td>0</td>
<td>77.0 ± 6.8b</td>
<td>10.12 ± 0.71c</td>
<td>0.29 ± 0.03ab</td>
<td>2.98 ± 0.45b</td>
</tr>
<tr>
<td>40</td>
<td>79.0 ± 2.0b</td>
<td>16.08 ± 0.34a</td>
<td>0.31 ± 0.04ab</td>
<td>4.98 ± 0.73a</td>
</tr>
<tr>
<td>80</td>
<td>71.0 ± 8.9b</td>
<td>14.55 ± 2.31ab</td>
<td>0.33 ± 0.02a</td>
<td>4.77 ± 0.52a</td>
</tr>
<tr>
<td>120</td>
<td>70.0 ± 7.7b</td>
<td>12.96 ± 1.38b</td>
<td>0.25 ± 0.03b</td>
<td>3.23 ± 0.64b</td>
</tr>
</tbody>
</table>

GP: germination percentage, GI: germination index, SFW: seedling fresh weight, VI: vigor index

Seeds were soaked in 0, 40, 80, and 120 mM H\textsubscript{2}O\textsubscript{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≤0.05

Table 2. Effects of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) seed treatment on pigment composition of B. ceiba after heat shock.

<table>
<thead>
<tr>
<th>H\textsubscript{2}O\textsubscript{2} (mM)</th>
<th>a (mg/g)</th>
<th>b (mg/g)</th>
<th>a+b (mg/g)</th>
<th>a/b</th>
<th>Carotenoid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.71 ± 0.84a</td>
<td>4.60 ± 0.43a</td>
<td>17.30 ± 1.24a</td>
<td>2.77 ± 0.13b</td>
<td>2.10 ± 0.07a</td>
</tr>
<tr>
<td>0</td>
<td>11.38 ± 1.07a</td>
<td>3.58 ± 0.42b</td>
<td>14.96 ± 1.43a</td>
<td>3.19 ± 0.23a</td>
<td>2.01 ± 0.19a</td>
</tr>
<tr>
<td>40</td>
<td>12.81 ± 1.50a</td>
<td>4.20 ± 0.37ab</td>
<td>17.01 ± 1.87a</td>
<td>3.05 ± 0.08a</td>
<td>2.22 ± 0.29a</td>
</tr>
<tr>
<td>80</td>
<td>11.84 ± 1.63a</td>
<td>3.88 ± 0.45ab</td>
<td>15.72 ± 2.08a</td>
<td>3.05 ± 0.07a</td>
<td>2.07 ± 0.29a</td>
</tr>
<tr>
<td>120</td>
<td>8.64 ± 1.09b</td>
<td>2.78 ± 0.34c</td>
<td>11.43 ± 1.43b</td>
<td>3.11 ± 0.02a</td>
<td>1.64 ± 0.19a</td>
</tr>
</tbody>
</table>

Seeds were soaked in 0, 40, 80, and 120 mM H\textsubscript{2}O\textsubscript{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≤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 unseeded control (Table 2). Chlorophyll a/b and carotenoid of seedlings from seeds pretreated with different concentrations of H\textsubscript{2}O\textsubscript{2} did not differ from those of seeds 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\textsubscript{2}O\textsubscript{2} but decreased by 24.1%, 22.2%, and 23.6% respectively, for seedlings from seeds pretreated with 120 mM of H\textsubscript{2}O\textsubscript{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 unseeded control (Table 3). The Fv/Fm, Y(II), qP, and ETR increased firstly for seedlings from seeds pretreated with 40-80 mM of H\textsubscript{2}O\textsubscript{2}, and then decreased for seedlings from seeds pretreated with 120 mM of H\textsubscript{2}O\textsubscript{2} compared to the seedlings without seed treatment. Y(II), qP, and ETR of seedlings from seeds pretreated with 80 mM of H\textsubscript{2}O\textsubscript{2} increased over those of the seedlings from untreated seeds, but did not differ from those of the unseeded control. The Y(NO) did not vary among the seedlings with and without seed treatments and the unseeded control.

MDA content: After heat shock, MDA of seedlings from untreated seeds did not differ from that of the unseeded 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\textsubscript{2}O\textsubscript{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 unseeded 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\textsubscript{2}O\textsubscript{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 from seeds pretreated with 80 and 120 mM of H\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{2} concentration relative to that of seedlings from untreated seeds, but only the proline content of seedlings with 120 mM of H\textsubscript{2}O\textsubscript{2} seed treatment increased significantly compared to that of the seedlings without seed treatment.
Table 3. Effects of hydrogen peroxide (H$_2$O$_2$) seed treatment on chlorophyll fluorescence characteristics of B. ceiba after heat shock.

<table>
<thead>
<tr>
<th>H$_2$O$_2$ (mM)</th>
<th>Fv/Fm</th>
<th>NPQ</th>
<th>qP</th>
<th>Y(II)</th>
<th>Y(NPQ)</th>
<th>Y(NO)</th>
<th>ETR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.76 ± 0.01a</td>
<td>0.42 ± 0.08d</td>
<td>0.73 ± 0.03a</td>
<td>0.50 ± 0.03a</td>
<td>0.14 ± 0.02c</td>
<td>0.35 ± 0.03a</td>
<td>52.66 ± 3.45a</td>
</tr>
<tr>
<td>0</td>
<td>0.72 ± 0.03b</td>
<td>0.50 ± 0.20c</td>
<td>0.51 ± 0.01c</td>
<td>0.33 ± 0.20b</td>
<td>0.22 ± 0.10b</td>
<td>0.45 ± 0.12a</td>
<td>33.43 ± 1.29b</td>
</tr>
<tr>
<td>40</td>
<td>0.75 ± 0.02ab</td>
<td>0.61 ± 0.23b</td>
<td>0.59 ± 0.03b</td>
<td>0.38 ± 0.01ab</td>
<td>0.23 ± 0.06b</td>
<td>0.39 ± 0.06a</td>
<td>38.63 ± 0.93b</td>
</tr>
<tr>
<td>80</td>
<td>0.74 ± 0.01ab</td>
<td>0.41 ± 0.13d</td>
<td>0.78 ± 0.03a</td>
<td>0.53 ± 0.01a</td>
<td>0.14 ± 0.03c</td>
<td>0.34 ± 0.03a</td>
<td>53.40 ± 1.42a</td>
</tr>
<tr>
<td>120</td>
<td>0.71 ±0.01b</td>
<td>0.71 ± 0.13a</td>
<td>0.36 ± 0.06d</td>
<td>0.21 ± 0.12b</td>
<td>0.32 ± 0.04a</td>
<td>0.47 ± 0.10a</td>
<td>21.17 ± 12.55c</td>
</tr>
</tbody>
</table>


Seeds were soaked in 0, 40, 80, and 120 mM H$_2$O$_2$ 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≤0.05.

Table 4. Effects of hydrogen peroxide (H$_2$O$_2$) seed treatment on activities of antioxidant enzymes of B. ceiba after heat shock.

<table>
<thead>
<tr>
<th>H$_2$O$_2$ (mM)</th>
<th>SOD (U/g)</th>
<th>POD (U/min/mg)</th>
<th>CAT (U/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>172.31 ± 17.61a</td>
<td>14.25 ± 5.55a</td>
<td>17.09 ± 0.42c</td>
</tr>
<tr>
<td>0</td>
<td>91.00 ± 7.16b</td>
<td>14.99 ± 2.28a</td>
<td>32.88 ± 5.42ab</td>
</tr>
<tr>
<td>40</td>
<td>118.81 ± 18.71b</td>
<td>13.79 ± 2.56a</td>
<td>32.66 ± 2.75ab</td>
</tr>
<tr>
<td>80</td>
<td>161.02 ± 21.83a</td>
<td>17.50 ± 7.58a</td>
<td>39.35 ± 4.31a</td>
</tr>
<tr>
<td>120</td>
<td>113.09 ± 8.94b</td>
<td>18.84 ± 4.23a</td>
<td>31.96 ± 4.15b</td>
</tr>
</tbody>
</table>

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

Seeds were soaked in 0, 40, 80, and 120 mM H$_2$O$_2$ 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≤0.05.

Discussion

Effects of H$_2$O$_2$ 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$_2$O$_2$ is a major ROS which play important 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$_2$O$_2$, respectively, increased after heat shock. Our results suggest that pretreatment of seeds with 40 to 80 mM of H$_2$O$_2$ increases germination rate of B. ceiba after heat shock. However, would H$_2$O$_2$ have similar effects in non-heat stressed seeds of B. ceiba is not clear. Some other studies also showed that exogenous application of H$_2$O$_2$ 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).

Fig. 1. Effects of hydrogen peroxide (H$_2$O$_2$) seed treatment on malondialdehyde (MDA) content of B. ceiba after heat shock. Seeds were soaked in 0, 40, 80, and 120 mM H$_2$O$_2$ 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≤0.05.

Effects of H$_2$O$_2$ seed treatment on the response of B. ceiba seedlings to heat shock: Besides seed germination, the metabolic processes of seedlings arising from primed seeds are affected by seed priming under various stresses (Jisha et al., 2013; Bhanuprakash & Yogeesh, 2016).
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$_2$O$_2$ seed treatment after heat shock indicated that chlorophyll a might be less susceptible to degradation than chlorophyll b. Compared to the seedlings without H$_2$O$_2$ seed treatment, pigment content and composition of seedlings from seeds pretreated with low concentrations of H$_2$O$_2$ did not change after heat shock, but chlorophyll content of seedlings from seeds pretreated with 120 mM of H$_2$O$_2$ decreased. Our results suggest that the effect of H$_2$O$_2$ on pigment content is related to its concentration, and pretreatment of seeds with high concentrations of H$_2$O$_2$ induces oxidative stress to seedlings of B. ceiba and promotes chlorophyll degradation. Similarly, Gechev et al. (2002) reported that the role of H$_2$O$_2$ was dependent on the dose of H$_2$O$_2$ applied, and high concentrations of H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ and then decreased for seedlings with 120 mM of H$_2$O$_2$ seed treatment compared to those of the seedlings without seed treatment. It is obvious that the role of H$_2$O$_2$ is concentration-dependent and a proper concentration of H$_2$O$_2$ can promote heat tolerance of B. ceiba at some extent. The Y(NPQ) and NPQ showed that MDA contents with 40 and 120 mM of H$_2$O$_2$ seed treatments but did not change for seedlings from seeds pretreated with 80 mM of H$_2$O$_2$ compared to those of the control. This trend might be due to the fact that seedlings with 40 and 120 mM of H$_2$O$_2$ seed treatments were heat stressed and could avoid further damage through thermal dissipation; however, seedlings from seeds pretreated with 80 mM of H$_2$O$_2$ 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$_2$O$_2$ did not have effect on MDA content. However, correlation analyses showed that MDA contents 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$_2$O$_2$ treatment (Gechev et al., 2002; Li et al., 2011). The SOD activities of seedlings from seeds pretreated with 80 mM of H$_2$O$_2$ increased compared to that of the seedlings without seed treatment, and the CAT activities of seedlings with 80 mM of H$_2$O$_2$ 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$_2$O$_2$ is conducive to protect seedlings of B. ceiba against oxidative stress induced by heat shock. Similar results were reported that H$_2$O$_2$ 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 cellular redox balance of stressed plants (Mahajan & Tutela, 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ increased heat tolerance of *B. ceiba* during seed germination and early seedling growth. However, a higher concentration of H$_2$O$_2$ at 120 mM induced chlorophyll degradation and photoinhibition. In application, seeds of *B. ceiba* can be primed with an appropriate concentration of H$_2$O$_2$ to improve seedling survival under high temperature stress. As this study was focused on effects of H$_2$O$_2$ on seed germination and seedling characteristics after heat shock, how did seed priming with H$_2$O$_2$ affect seed germination and seedling growth of *B. ceiba* was 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$_2$O$_2$ 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.

**References**


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