Effect of desiccation on antioxidant enzymes activity of recalcitrant tea (Camellia sinensis L.) seeds

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ABSTRACT: Tea (Camellia sinensis (L.) O. Kuntze) is the most popular beverage consumed by over two thirds of the world’s population. Tea seed is believed to be recalcitrant based on its sensitivity to chilling or drying stress. Desiccation interferes with metabolic processes of recalcitrant seeds causing an increase in potentially harmful reactive oxygen species (ROS) and alterations in cytosolic redox statues. It investigated changes antioxidant enzyme activity of recalcitrant seed of 5 genotypes of tea in response desiccation. Mature tea seeds were harvested from plants grown in the tea field of Fashalam tea research station in IRAN. Seed were desiccated at 15% relative humidity at 15°C at different intervals of time. Measuring total protein, and enzymes activity assay such as ascorbate peroxides (APX), superoxide dismutas (SOD), peroxidase (POD) and catalase (CAT) carried out in 5 times with prolonged desiccation in factorial completely randomized design in three replicates. Results showed that desiccation treatment dramatically increased activities of antioxidant enzymes like APX and SOD. Increased antioxidant, CAT partially assuaged desiccation damage to tea seed, resulting in improved germination rates. APX and CAT activities in tea seed embryos were slightly increased with prolonged desiccation, while SOD and POD activities decreased. It proposes that desiccation causes an over-accumulation of ROS that are not efficiently scavenged by increased levels of antioxidant enzymes. High levels of ROS alter the redox status and are detrimental to seed viability. Reducing ROS to appropriate concentrations in an efficient way to reduce desiccation damage and improve germination rates of recalcitrant seeds.

Key words: antioxidant enzymes, recalcitrant seeds, Camellia sinensis

INTRODUCTION

Plant seeds can be classified as recalcitrant and orthodox based on their storage properties. Recalcitrant seeds are sensitive to chilling and drying treatment. Such seeds can maintain high water content and high metabolic rates from the time of seed maturation until germination. Orthodox seeds are tolerant to desiccation stress and can be well preserved under low temperatures. Another type of seed namely, intermediate seeds have also been reported whose property falls between recalcitrant and orthodox types (Roach et al. 2008; Hill et al. 2010). Recalcitrant seeds cannot be stored at low temperatures and low moisture content. Tea extracts, mainly composed of soluble polysaccharide, protein, amino acid, caffeine, and polyphenol, determines the quality of processed tea. Environmental stress can affect these components and then influence the sensory quality (flavour, taste and colour) of tea infusion (Chen et al. 2010). Desiccation interferes with metabolic processes of recalcitrant seeds causing an increase in potentially harmful reactive oxygen species (ROS) including hydrogen peroxide (H₂O₂), superoxide’s, singlet oxygen and hydroxyl radicals, but there is little information available regarding how ROS are regulated in seeds susceptible to drying stress. The toxic ROS may disrupt membrane integrity via peroxidation of membrane lipids (Roach et al. 2008; Ahmad et al., 2010; Bailly, 2007). Tea polyphenols may act as antioxidants by scavenging reactive oxygen and nitrogen species and by chelating redox-active transition metal ions, and may also act indirectly as antioxidants through, among other mechanisms, the inhibition of “pro-oxidant” enzymes and induction of antioxidant enzymes. In this paper, we report the role of some enzymes in the regulation of ROS metabolism using physiological method. Reducing ROS to appropriate concentrations via ascorbate–glutathione pathway enzymes is an efficient way to improve germination rates of recalcitrant seed after desiccation stress. This will help in development of new methods for recalcitrant seed storage and conservation.
MATERIALS AND METHODS

Mature tea seeds of five genotypes (DN, 100, 399, 437, 1146) were harvested from plants grown in the tea research station of Iran (Fashalam) in 2012. Pericarp of the seed was removed prior to desiccation. Seeds were desiccated at 15% relative humidity and 15°C at different intervals of time. The fresh seeds under 70% relative humidity and 30°C were used as the control. Moisture content of seeds was determined gravimetrically by oven drying for 17 h at 103°C by the method given by Roach et al. (2010). All measurements were done on 5 samples of 10 seeds of equal size. Tea seeds were ground with a pestle in an ice-cold mortar with 2.5 ml of phosphate buffer (pH 7.0, 0.05 M). Homogenate was filtered through four layers of cheesecloth and then centrifuged at 4°C for 20 min at 15,000g. The supernatant was collected and used for enzymatic activity and protein content assays. SOD activity was determined according to the method of Donahue et al. (1997). One enzyme unit of SOD was defined as the amount of enzyme that inhibited 50% NBT by photoreduction and the activity was expressed in enzyme units (U/mg protein). The absorbance of the reaction product was measured at 560 nm. Activities of catalase (CAT) and ascorbate peroxidase (APX) were measured according to the method of Knorzer et al. (1996). For APX, the assay depends on decreased absorbance at 290 nm as ascorbate is oxidized. For CAT, the decomposition of H₂O₂ is followed by a decline in absorbance at 240 nm. Activities of APX and CAT were expressed in enzyme units per mg protein. Peroxidase (POD) activity was measured by the method of Huang et al. (2010). The oxidation of guaiacol was measured by an increase in absorbance at 470 nm.

RESULTS

ANOVA results showed that the SOD between genotypes and between times difference are significant at 1% level. Interaction different between genotypes × times, there were significant difference at 5% level. The APX and POD showed that between genotypes, between times and between genotypes × times difference are significant at 1% level. Also, the CAT activity in tea seeds between genotypes and between times was significant difference at 1% level (Tab 1).

![Bar chart showing effect of desiccation on SOD activity in 5 genotypes of tea](image)

Table 1. Analysis of variance

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>MS SOD</th>
<th>APX</th>
<th>POD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>2</td>
<td>0.0002</td>
<td>169.00</td>
<td>0.013</td>
<td>0.185</td>
</tr>
<tr>
<td>Genotypes</td>
<td>4</td>
<td>0.509</td>
<td>4723.68</td>
<td>21.404</td>
<td>0.980</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.038</td>
<td>11.80</td>
<td>0.036</td>
<td>0.049</td>
</tr>
<tr>
<td>Times</td>
<td>4</td>
<td>0.787*</td>
<td>15064.38*</td>
<td>23.749*</td>
<td>1.325*</td>
</tr>
<tr>
<td>Genotypes × Times</td>
<td>16</td>
<td>0.068</td>
<td>495.25</td>
<td>0.659</td>
<td>0.101</td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>0.029</td>
<td>25.64</td>
<td>0.095</td>
<td>0.071</td>
</tr>
<tr>
<td>Grand Mean</td>
<td>1</td>
<td>1.322</td>
<td>63.32</td>
<td>5.647</td>
<td>1.546</td>
</tr>
</tbody>
</table>

Figure 1. Effect of desiccation on SOD activity in 5 genotypes of tea.
Based on results, we found that desiccation treatment induced a rapid accumulation of antioxidant enzymes including APX, SOD and additional proteins in seed embryos. Increased in \( \text{H}_2\text{O}_2 \) is accompanied by increase in antioxidant enzyme activities including CAT, SOD, APX and POD under desiccation treatment. APX and CAT activities in some genotypes of tea were slightly increased with prolonged desiccation, while SOD and POD activities decreased in some genotypes e.g. 399, 1146 with prolonged desiccation (Fig 1-4). The amount of SOD and APX activities were significant differences between genotypes and promising clone 100 with the highest activity and 399 genotype with the lowest activity were grouped (Fig 1& 2). Maximum enzyme activities were observed at 40 hours after desiccation treatment and promising clone 100 showed that highest activity of SOD, APX and CAT in 80 hours after treatment. The amount of POD and CAT activities between genotypes...
were significant, similarly. Also, promising clone 100 showed the highest activity of these enzymes while the lowest activities were fluctuating between genotypes 437 and 399 (Figure 3 & 4).

**DISCUSSION**

The characteristic nature of recalcitrant seeds is their loss of germination capacity upon desiccation or chilling treatments. The detailed dynamic changes of proteins in response to these stresses are poorly understood. Proteins related to defense response showed remarkable changes after desiccation treatment. Eventually, a series of defense responding proteins begin to participate in the process (e.g., seed enzymes related to ROS metabolism) (Chen et al. 2010). With prolonged desiccation treatment accumulation of these proteins declined. It is possible that relatively longer desiccation period caused irreversible damage to embryo protein systems that result in the loss of seed viability (Chen et al. 2010). ROS, especially H$_2$O$_2$, serves as an essential signal in multiple aspects of physiological processes (Neill et al. 2002). Recent evidence indicates that it plays a role in drying or chilling intolerance and cell death in recalcitrant seeds (Roach et al. 2008, 2010). It plays numerous beneficial roles such as in stress signaling, cell wall-strengthening and activation of defense related genes; however, over-accumulation of ROS becomes toxic to normal plant metabolism (Song, 2004; Jaspers et al. 2010). Based on our results, we conclude that prolonged periods of desiccation because rapid accumulation of ROS, which cannot be efficiently removed by increased levels of antioxidant enzymes. Subsequently, the imbalanced redox status leads to over-accumulation of ROS, which extensively suppress seed viability. Efficient removal of accumulated ROS by scavengers can reverse the inhibitory effect of desiccation stress on seed germination (Wu et al. 2009). Genotypes capable of defense against free radicals, which were also very different, that returns their genetic efficiency. Also, in all enzymes scavenger of free radical, promising clone 100 can be tolerated with higher efficiency during periods of desiccation. This feature can be exploited in future breeding programs. With increasing time, SOD activity increased and genotypes 437, 100 and DN had similar behavior. But the genotypes 399 and 1146 were increased at the first level and then they decreased. The same trend was also observed in APX. But in POD, all the genotypes with increasing time, showed increase in activity but after that POD activity decreased. The CAT activity, genotypes had similar increasing trend as promising clone 100, genotypes 1146 and DN 399 and 437 were similar. The time interval between the minimum (0 h) to the maximum time (80 h) only in extreme APX activity assay was significant completely. In the case of other enzymes, this distance were less and non-significant. These results presented the important role of the antioxidant enzymes activity against the desiccation stress. In conclusion, the survey demonstrated that reducing ROS to appropriate concentrations in an efficient way to reduce desiccation damage and improve germination rates of recalcitrant seeds.
REFERENCES


