EXOGENOUS ABSCISIC ACID INCREASES RESISTANCES AGAINST ABIOTIC STRESS AND IMPROVE FRUIT QUALITY OF GRAPE

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ABSTRACT

The objective of this study was to determine the stress resistance response and fruit quality of grape to exogenous abscisic acid (ABA). Five concentrations (100, 150, 200, 250 and 300 mg/L) of ABA were tested to evaluate its protective activity in grape leaves and fruit quality of 3-year-old ‘Red Globe’ grape, cultivated under the plastic rain shelter. It was found that addition of ABA significantly decreased the level of electrolyte leakage and lipid peroxidation, enhanced the activities of superoxide dismutase, peroxidase, proline content and soluble sugars. Meanwhile, exogenous ABA improved the anthocyanin, soluble solids, titrable acidity and AsA. Nevertheless, it had less effect on fruit shape index and weight of grape fruit (bunch and single berry). It was concluded that exogenous ABA may increase stress resistances and improve fruit quality of grape.

Key words: ABA; Grape; Stress resistance; Fruit quality.

INTRODUCTION

Grape is one of the most important and widely cultivated fruit crop around the world. China, with about 14% of the world production in 2012 (data from the United Nations Food and Agriculture Organization, FAO), is the largest contributor to grape (Vitis vinifera) industry in the world. The grape production of China has increased by more than 200% since 2000 (data from the International Organization of Vine and Wine, OIV). Some southern provinces have become the largest area of grape industry growth in China, Zhejiang, Jiangsu and Sichuan for example (data from the Ministry of Agriculture of China). However, many local vineyards in southern China were confronted with low light, high temperature and humidity in growing season, which adversely affect the growth, color development and stress resistance of grapes.

Abscisic acid (ABA) has been known as a stress induced phytohormone. ABA plays essential roles in the plant’s response to abiotic stresses, which include chilling, salinity, high temperature and drought (Hsu and Kao, 2003). ABA induces stomatal closing, thus reduces excess water loss from leaves and consequently water uptake from roots (Schroeder et al., 2001). Adaptation of the plants to such environmental stresses is associated with increase in ABA levels (Zhu, 2002).

Exogenous ABA application has been used on various vegetables exposed to different environmental stresses (Raghavendra et al., 2010; Jia et al., 2011; Nakashima and Yamaguchi-Shinozaki, 2013). It has been observed that application of exogenous ABA on leaves accumulate endogenous ABA and enhance activities of antioxidant enzymes including glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Nakashima and Yamaguchi-Shinozaki, 2013). Moreover, reactive oxygen species (ROS) could be removed by exogenous ABA to enhance the cell membrane stability and alleviate stress damage to plants (Parent et al., 2009; Wang et al., 2011; Nese et al., 2012; Zhang et al., 2014). Overall plant responses to ABA may vary according to concentration, application method, plant species, and stage of plant development (Ferrara et al., 2013; Hussain et al., 2014).

In addition, ABA also participates in the initiation of ripening and related changes in grape development (Peppi and Fidelibus, 2008; Koyama et al., 2010). It has been demonstrated that exogenous application of ABA increases the anthocyanins in different grapes varieties, resulting in improved color and berry quality (Lurie et al., 2009; Lacampagne et al., 2009; Sandhu et al., 2011; Ferrara et al., 2013).

While, many studies indicate that exogenous ABA may enhance stress resistance in various crop species, studies are seriously lacking on the evaluation of grape responses to low light, high temperature and humidity stress by application of exogenous ABA. Also, many studies had investigated the effect of exogenous application of ABA on color of berry; however the effects of ABA on other fruit quality characteristics have not been investigated. The objective of this study was to evaluate the role of exogenous ABA in stresses response and fruit quality of grape. The results will help in developing practical guidelines to mitigate the negative effects of high temperature and humidity stress on grape grown in southern China.
**MATERIALS AND METHODS**

**Plant material and experimental design:** The study was performed on 3-years-old ‘Red Globe’ grape (*Vitis vinifera* L.) with a bilateral cordon training system trellised to a three-wire vertical system on 2012, in a commercial table grape vineyard located in the territory of Yong-an town (N30°24′, E103°59′), Chengdu, China. Vine rows were cultivated under the plastic rain shelter and planting density was 3 m between rows and 1 m between vines. Fertilizer additions, pest control, and other vineyard operations were conducted according to local practices.

A randomized complete blocks design (RCBD) was used with three blocks and six treatments, and each treatment in the block consisted of four grapevines. In each treatment, exogenous ABA was applied at 0, 100, 150, 200, 250 and 300 mg/L. The solutions of ABA were made by dissolving ABA in water that contained 0.1% (V/V) of Tween 20 as a wetting agent. The control solution contained water with Tween 20. At the start of berry veraison in Chengdu region, ABA was applied on July 10 directly to the leaves and berries with a handheld sprayer until runoff. Leaves were collected after 0, 10, 20, 30, 40 and 50 days of ABA treatment, while fruits were sampled when the last collection of leaf samples. All samples were immediately frozen in liquid nitrogen and then stored at -70 °C until use.

**Lipid peroxidation and electrolyte leakage assays:** Lipid peroxidation was determined as the content of all 2-thiobarbituric acid reactive substances and expressed as equivalents of malondialdehyde (MDA), as described by Heath and Packer (1968).

Electrolyte leakage (EL) was determined by following the method of Dionisio-Sese and Tobita (1998). For this purpose ten leaf discs (5 mm in diameter) were placed in test tubes containing 10 ml distilled water. The tubes were incubated in a water bath at room temperature for 2 h and the initial electrical conductivity of the medium (EC1) was recorded using an electrical conductivity analyzer (DDS-307, Shanghai Precision Scientific Instrument Co., Ltd, China). The samples were autoclaved at 100 °C for 20 min to release all electrolytes, then, cooled to 25 °C. The final electrical conductivity (EC2) was recorded. The EL was calculated using the formula: EL= (EC1/EC2)*100.

**SOD and POD activities:** Frozen leaves (0.5 g) were homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, 3 mM 2-mercaptoethanol, and 2% (w/v) polyvinylpolypyrrolidone in a chilled mortar and pestle. The homogenate was centrifuged at 16,000 g for 30 min at 4°C and the supernatant was used for the following enzyme assays.

The SOD activity was assayed by the nitroblue tetrazolium (NBT) method (Dhindsa et al., 1981). The reaction mixture (3 ml) contained 50 mM Na-phosphate buffer (pH 7.3), 13 mM methionine, 75 mM NBT, 0.1 mM EDTA, 4 mM riboflavin, and enzyme extract (0.2 ml). The reaction was started by the addition of riboflavin under fluorescent lamps (160 mol m⁻² s⁻¹). The reaction was allowed to proceed for 5 min and was then stopped by switching off the light. The absorbance was measured at 560 nm. Blanks and controls were run in the same manner but without illumination and enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions.

The POD activities were assayed following the method of Chance and Maehly (1955) with some modification. The POD reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.8), 25 mM guaiacol, 200 mM H₂O₂, and 0.5 ml enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30s. One unit of POD activity was defined as an absorbance change of 0.01 units/min.

**Proline and soluble sugar content:** The colorimetric method, using acidic ninhydrin was followed to estimate proline contents (Bates et al., 1973). Leaf tissues were put into a tube with 4 ml 80% ethanol and the solution was collected (repeated twice). The solution was, then, heated for 30 min in a boiling water bath. After cooling, the solution was centrifuged for 10 min at 3000 rpm and the supernatant was collected to determine the soluble sugar by using anthranone colorimetry with glucose as the standard (Yuan et al., 2011).

**Total anthocyanin contents and fruit shape index:** Total anthocyanin content of the samples were determined using the pH-differential method described by Orak (2007). The fruit shape index was calculated as the length: diameter ratio for each fruit. The mean of three fruits was calculated to estimate the central tendency of each fruit size phenotype character.

**Soluble solids and titrable acidity content:** The Soluble solids content (SSC) was measured by a pocket refractometer, PAL-1 (ATAGO, Japan). Anthrone colorimetry method and acid base titrations were adopted to determine the content of total carbohydrate and titrable acidity (Hao et al., 2006).

**AsA content:** Leaf sample (0.5g) was ground with a mortar and pestle in 2 mL of 0.5 M EDTA solution containing 3% trichloroacetic acid and centrifuged at 15,000 g for 10 min at 4°C. The supernatant was used for assays of the levels of ascorbic acid (AsA). The amount of AsA was estimated using the method of Foyer et al. (1976) and was expressed as mg/g FW.
Statistical analysis: Experiments were performed according to a completely randomized design. Data were expressed as mean ± standard errors (SE). All statistical analyses were performed with SPSS 17.0. One-way analysis of variance (ANOVA) and Duncan’s multiple range tests were used to determine the significance of the difference among samples, with a significance level of 0.05.

RESULTS

Membrane damage: The membrane damage was investigated by monitoring MDA content and EL. The MDA content of leaves in all treatments showed comparable pattern, which increased drastically from 0 DAV, peaked on 30DAV, then decreased until 50DAV. However the accumulations of MDA in the treatments of exogenously applied ABA were always lower than that of control treatment (Figure. 1A). Leaves treated with 250 and 300 mg/L had lowest MDA content in all ABA-treated groups. The change pattern of EL was similar with MDA during the treatment, but the change was not as great as that for MDA (Figure. 1B). These results indicated exogenously applied ABA can decrease membrane damage for leaves in summer high temperature.

Activities of antioxidant enzymes: The activities of antioxidant enzymes involved in scavenging ROS changed significantly with ABA treatment. Changes of SOD and POD activities were similar in the leaves, rising gradually to a peak on 20DAV and decreasing gently with prolonged treatment. The ABA-treated leaves had much higher SOD and POD activities than that of the control (Figure. 2A, 2B). The highest SOD and POD activity were observed in treatment spayed by 250 and 300 mg/L exogenous ABA during the whole experimental process. These results suggest that ABA application may increase the activation of SOD and POD in scavenging ROS under high temperature during summer.

Proline and soluble sugar concentrations: During experimental process, concentrations of proline and soluble sugars increased continuously and drastically, peaking on 30DAV, then decreased gently by 50DAV, as compared to the concentrations of proline and soluble sugars on 0DAV (Figure. 3A, 3B). The accumulation of proline and soluble sugar with ABA treatments were always higher than that of control treatment. The highest concentrations of proline and soluble sugar were observed in treatment spayed by 250 and 300 mg/L exogenous ABA during the whole experimental process. These results indicated the stimulation of exogenous ABA effects on proline and soluble sugar synthesis under high temperature condition.

Changes in visual quality: Visual quality changes in response to exogenous ABA were detected by weight of bunch and berry, fruit shape index and anthocyanin content. Compared with the control, there were no significantly increase in weight of bunch and berry for ABA-treated fruits (Table 1). Among all ABA-treated groups, the anthocyanin content of fruit treated with 250 and 300 mg/L exogenous ABA had the highest level and was significant differences with other treatments. However, unlike other appearance quality indicators, ABA application had less effect on fruit shape index (Table 1).

Changes of interior quality: The soluble solids contents, titratable acid and AsA of grape berry were analysis to explore the influence of exogenous ABA on chemical fruit quality attributes. There were significant differences in all identified indexes in this experiment between ABA-treated groups and control. The contents of soluble solids and AsA in fruit treated with 250 and 300 mg/L exogenous ABA were lowest in all ABA-treated groups. These two treatments had significant differences with other treatments (Table 2). Compared with other treatments, treatment 5 (name of treatment) showed the lowest titratable acidity.
Figure 1. Effects of different exogenous ABA treatments on content of electrolyte leakage (A) and MDA (B). Each value is the mean of three independent experiments, and the vertical bars indicate standard error (SE) (n = 3).

Figure 2. Effects of different exogenous ABA treatments on activity of SOD (A) and POD (B). Each value is the mean of three independent experiments, and the vertical bars indicate standard error (SE) (n = 3).
Figure 3. Effects of different exogenous ABA treatments on content of proline (A) and soluble sugar (B). Each value is the mean of three independent experiments, and the vertical bars indicate standard error (SE) (n = 3).

Table 1. Effects of different exogenous ABA treatments on appearance quality.

<table>
<thead>
<tr>
<th>Concentration of exogenous ABA (mg/L)</th>
<th>Single panicle weight (g)</th>
<th>Weight of single fruit (g)</th>
<th>Fruit shape index</th>
<th>Anthocyanin (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1212.00±27.79a</td>
<td>13.38±1.7a</td>
<td>1.132±0.27a</td>
<td>127.50±12.00c</td>
</tr>
<tr>
<td>100</td>
<td>1230.00±28.83a</td>
<td>13.48±1.2a</td>
<td>1.135±0.12a</td>
<td>129.70±12.34c</td>
</tr>
<tr>
<td>150</td>
<td>1200.00±17.47a</td>
<td>13.61±1.9a</td>
<td>1.132±0.19a</td>
<td>130.03±21.67c</td>
</tr>
<tr>
<td>200</td>
<td>1200.00±12.00a</td>
<td>14.05±1.4a</td>
<td>1.130±0.14a</td>
<td>140.17±11.79b</td>
</tr>
<tr>
<td>250</td>
<td>1226.67±24.04a</td>
<td>14.11±1.7a</td>
<td>1.134±0.17a</td>
<td>148.50±15.94a</td>
</tr>
<tr>
<td>300</td>
<td>1228.00±20.84a</td>
<td>13.82±1.6a</td>
<td>1.130±0.24a</td>
<td>150.37±20.85a</td>
</tr>
</tbody>
</table>

Data represent means±SE of 3 replicate samples. Different letters indicate significant differences according to a Duncan’s multiple range test (P < 0.05).
DISCUSSION

Low light, high temperature and humidity in growing season are the major constrains of grape development in southern China. These stresses can negatively affect the growth, coloring and stress resistance of grapes (Mori et al., 2005; Sandhu et al., 2011).

Stress conditions, which induce membrane damage and leakage of electrolytes, increase the formation of reactive oxygen species (ROS) (Ma et al., 2008). MDA is a product of lipid peroxidation in cell membranes, and its content in vivo can indicate the extent of oxidative stress in plants and membrane homeostasis (Wang et al., 2011). Our results demonstrate that different stresses caused a dramatic increase in MDA content and EL grape leaves, indicating the presence of oxidative damage. However, exogenous applications of ABA were effective in lowering the levels of MDA and reducing electrical conductivity of leaves (Figure 1 and Figure 2). These results agreed with previous reports (Lu et al., 2008; Ma et al., 2008; Wang et al., 2011), which reported that exogenous ABA can alleviate oxidative stress induced by varied stress in growing season of grape.

The enhancement of antioxidant activities was often related to improvement of stress tolerance in plants. For example, a higher amount of ROS triggers up-regulation of the activity of antioxidant enzymes, which in turn protects plants from oxidative stress (Davey et al., 2000). The most important detoxifying enzymes to promote the scavenging of ROS include SOD, POD, and CAT (Danquah et al., 2014). The activities of SOD and POD in stressed leaves first increased, then decreased over time. The ABA-treated leaves have much higher SOD and POD activities than the control (Figure 3, 4). This suggests that such modulation in response to stress has an upper limit, and also implies that exogenous ABA can increase the antioxidant enzymes activities.

Proline and soluble carbohydrates are compatible solutes, which can increase cellular osmotic pressure (Redondo-Gomez, 2013) to resist abiotic or biotic stress. The high hydrophilicity of compatible solutes helps to maintain the turgor pressure and water content of cells which protect against water loss from leaves under abiotic stress (Yokota et al., 2006). The roles of ABA in proline accumulation were described by Verslues and Bray (2006). These authors demonstrated the dependency of proline accumulation not only on the ABA amount but also on the plant sensitivity, or competency, to respond to the ABA. Gurmani et al. (2011) reported that ABA was the most effective plant growth regulator in reducing Na+ and Cl- concentrations and Na+/K+ ratio, increasing K+ and Ca2+ concentrations, proline accumulation, soluble sugar content and grain yield. In the present study, similar results were observed: all the ABA-treated leaves had higher concentration proline (Figure 5) and soluble sugar (Figure 6) when compared to the control. It indicates that exogenous ABA can induce accumulation of proline and soluble sugars in leaves to resist different stresses.

It has been demonstrated that exogenous ABA application can induce anthocyanin accumulation in grape (Giribaldi et al., 2009; Wheeler et al., 2009). Our results showed that ABA significantly increased the anthocyanin production in ABA-treated plants. The possible reasons for the results may be ABA can up-regulate anthocyanin in grape by stimulating gene expression of enzymes in the anthocyanin biosynthetic pathway (Jeong et al., 2004; Koyama et al., 2010).

Weight of single panicle and single fruit did not show differences among ABA treatments (Table 2). Our results are in agreement with those of previous studies that reported that applications of S-ABA had little or no effect on grape weight (Cantin et al., 2007; Peppi et al., 2007).

Endogenous ABA plays an important role in plant growth. For example, ABA also participates in the initiation of ripening and related changes in grape development (Ferrara et al., 2013). In this study, all the treatments increased SSC and reduced TA in berries, compared to control. It is indicated that ABA may induced the maturation of berries, therefore, the content of SSC was increased and TA was reduced. Our results are in agreement with previous studies (Peppi et al., 2007; Peppi and Fidelibus, 2008). However, it has also

### Table 2. Effects of different exogenous ABA treatments on interior quality

<table>
<thead>
<tr>
<th>Concentration of exogenous ABA (mg/L)</th>
<th>Soluble solids (%)</th>
<th>Titratable acid (100ml)</th>
<th>AsA (mg/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.14±1.1d</td>
<td>0.58419±0.05a</td>
<td>5.40±0.23b</td>
</tr>
<tr>
<td>100</td>
<td>16.02±1.4bc</td>
<td>0.57797±0.03ab</td>
<td>6.04±0.39b</td>
</tr>
<tr>
<td>150</td>
<td>16.49±2.2b</td>
<td>0.54889±0.02bc</td>
<td>5.89±0.36b</td>
</tr>
<tr>
<td>200</td>
<td>15.79±2.5c</td>
<td>0.53504±0.02cd</td>
<td>6.13±0.25b</td>
</tr>
<tr>
<td>250</td>
<td>17.56±1.8a</td>
<td>0.50469±0.02cd</td>
<td>7.31±0.20a</td>
</tr>
<tr>
<td>300</td>
<td>17.18±1.6a</td>
<td>0.51742±0.01d</td>
<td>7.11±0.17a</td>
</tr>
</tbody>
</table>

Data represent means±SE of 3 replicate samples. Different letters indicate significant differences according to a Duncan’s multiple range test (P < 0.05).
found that applications of S-ABA had little or no effect on both grape soluble solids and titratable acidity (Jeong et al., 2004; Peppi et al., 2006). The difference in these parameters may be due to different varieties, application time and concentration and cultivation pattern.

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