Abscisic Acid Accumulation in Leaves and Cultured Cells during Heat Acclimation in Grapes

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Abstract. Heat tolerance and endogenous ABA levels in leaves and cultured grape cells (*Vitis* spp., cultivars Venus and Veeblanc) were evaluated during heat acclimation. Plants and cultured cells were acclimated at 38 and 36C, respectively. Heat tolerance increased rapidly after exposing plants or cells to acclimation temperatures, reaching a maximum after 10 to 16 hours and 10 to 12 hours for leaves and cultured cells, respectively. Free and bound ABA levels increased sharply during the first hour of heat acclimation, before leaves and cultured cells reached their maximum heat tolerance. The increase in ABA during heat acclimation was 2- to 3-fold that of the nonacclimated control, and the time of the ABA accumulation peak in tissue roughly corresponded to the maximum heat tolerance in leaves and cultured cells. Heat tolerance was induced in cultured cells by exogenous ABA application. Heat tolerance increased significantly after 24 hours of ABA application at 7.6 or 9.5 μM . The results suggest that ABA may be a factor in high-temperature acclimation and heat-tolerance induction in grapes. Chemical name used: abscisic acid (ABA).

ABA is believed to play a role in plant tolerance to extreme environmental conditions (Salisbury and Marinos, 1985; Zeevaart and Creelman, 1988). Endogenous ABA accumulation in response to environmental stress has been shown in many plant species (Daie and Campbell, 1981; Eze et al., 1983; Skriver and Mundy, 1990; Zeevaart and Creelman, 1988). Many studies have demonstrated ABA accumulation during cold acclimation of plants and cold tolerance induction by exogenous ABA application (Bomman and Jansson, 1980; Chen and Gusta, 1983; Reaney et al., 1989). A similar ABA response has been noted with chilling temperatures and water stress (Hiron and Wright, 1973; Loveys and Kriedemann, 1973; Markhart, 1984). ABA may reduce chilling injury (Bomman and Jansson, 1980; Rikin and Richmond, 1976; Rikin et al., 1979) and the deleterious effects of water stress in plants (Mizrahi et al., 1974).

Although ABA accumulates in plants in

response to high temperatures (Daie and Campbell, 1981; Daie et al., 1981), the role of ABA in heat acclimation, tolerance, and injury is not clear. Despite reports of increased ABA levels in plants exposed to high temperatures, little is known about the time-course ABA accumulation in plants during heat acclimation.

In the present study, we report ABA accumulation in leaves and suspension-cultured grape cells during heat acclimation and show the effects of exogenous ABA application on the heat tolerance of cultured grape cells.

Plant materials. Stem cuttings from 'Venus' and 'Veeblanc' grape hybrids were collected from the Ashland Horticultural Research Farm, Kansas. The cuttings were rooted in a greenhouse under mist in pots containing a mixture of 1 peatmoss : 1 perlite (v/v). The rooted cuttings were repotted into larger pots and grown in the greenhouse at $25 \pm 3\text{C}$ and 80% relative humidity for 8 to 10 weeks.

Suspension-cultured cells. Callus cultures were initiated from 1- to 2-cm-long stem segments. Explants were surface-sterilized with aqueous 0.25% sodium hypochlorite for 20 min and rinsed three times in sterile distilled water. Explants were grown on Schenk and Hildebrandt (SH) solid medium (Schenk and Hildebrandt, 1972) containing 3.7 mg 1-

naphthaleneacetic acid (NAA) and 1.08 mg kinetin/liter. Suspension-cultured cells were initiated from 7-week-old callus using SH liquid medium (same as above without agar). The cultures were maintained by subculturing 10 ml of the suspension into 20 ml of fresh medium in 125-ml Erlenmeyer flasks every 10 days. The cultures were grown at 25C on an orbital shaker at 90 rpm. All experiments consisted of three replications.

Plant acclimation. The optimum temperature and duration of heat acclimation were determined in a preliminary experiment. For heat acclimation, 2-month-old plants were transferred to growth chambers and kept at 38C for 3 days.

Moisture content of the medium was monitored and maintained near field capacity. Suspension cultures were kept at 36C for 24 h. Leaf and cell samples were collected every 4 h during the first 24 h of heat acclimation and then every 12 h for the remaining period. Heat tolerance of cultured cells was determined by subjecting the samples to 42C for various lengths of time. Samples were then held for 12 h at room temperature before measuring conductivity using a conductance meter (YSI 32, Ohio). Conductivity was measured again after subjecting the samples to 80C for 30 min and then keeping them at room temperature for 12 h. The percent electrolyte leakage was plotted against the exposure time, and the transition midpoint was considered the lethal exposure time.

Endogenous ABA determination. Samples of fully expanded leaves and cultured cells were collected before and during acclimation at 0.5, 1, and 6 h, and subsequently every 6 h for the first 24 h, and then every 12 h for the

Fig. 1. Changes in heat tolerance in grape leaves during heat acclimation. Leaves of 'Venus' (O) and 'Veeblanc' (●) were subjected to 42C, and lethal exposure time was determined based on the electrolyte leakage test. The plants were acclimated at 38C.

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remaining period. Samples were dipped immediately in liquid nitrogen and then freeze-dried. The freeze-dried samples were ground to a fine powder using a Wiley mill. Procedures of Takeda and Crane (1980) were used for ABA extraction and purification. Free and bound ABA were quantified following the procedure of Durley et al. (1982). We used a dual-pump gradient high-performance liquid chromatograph (HPLC) (ISCO, Nebraska) equipped with a 250-mm $\times$ 4.6-mm Econosil reverse-phase C18 column (Alltech Assoc., Illinois).

Exogenous ABA application and heat tolerance. Suspension cultures (50 ml) were incubated with 3.8, 7.6, and 9.5 μM of (+)-ABA (Sigma, St. Louis) for 24 h. The exposure time was based on the maximum response to ABA in preliminary experiments. Cells were centrifuged and washed three times with 0.3 M mannitol before being suspended in $\approx$ 10 ml of 0.3 M mannitol. Heat tolerance of cultured cells was determined using the procedure described above.

Fig. 2. Changes in heat tolerance in suspension-cultured cells of grapes during heat acclimation. The cultured cells of 'Venus' (○) and 'Veeblanc' (●) were subjected to 42°C, and lethal exposure time was determined based on the electrolyte leakage test. The cells were acclimated at 36°C.

Fig. 3. Changes in free and bound endogenous ABA levels in grape leaves during heat acclimation. 'Venus' (A) and 'Veeblanc' (B) plants were acclimated at 38°C, and free (●) and bound (○) ABA levels in leaves were determined during acclimation.

Leaf heat tolerance increased and reached a maximum in 10 to 16 h and showed little change afterward (Fig. 1). 'Venus' was more heat tolerant than 'Veeblanc' before and during heat acclimation. The increase in lethal exposure time at 42°C was 50 to 60 min in response to acclimation. The acclimation response of cultured cells was similar to that of leaves (Fig. 2). The cells reached a maximum heat tolerance in 10 to 12 h during acclimation. These results are consistent with those from previous studies on many plant species (Chen et al., 1982; Lester, 1985; Santarius and Muller, 1979).

Free and bound ABA levels were monitored in leaves during acclimation. A sharp increase in free ABA was observed immediately after subjecting the plants to acclimation (Fig. 3). The free ABA level in leaves increased $\approx$ 3-fold and reached a maximum between 6 to 18 h during acclimation. The initial peak of free ABA level was followed by a large decline; however, the free ABA levels were much higher in leaves during acclimation than in the nonacclimated control. Studies by Daie and Campbell (1981) showed that ABA accumulated in tomato (*Lycopersicon esculentum* Mill) and pea (*Pisum sativum* L.) (Daie et al., 1981) plants after exposure to 35 to 48°C for 24 h.

Changes in bound and free ABA levels of leaves were similar during acclimation (Fig. 3). Bound ABA levels peaked slightly sooner, and the initial peak for bound ABA was smaller than that for free ABA. Bound ABA levels were higher in acclimated leaves than in the nonacclimated control.

The changes in free and bound ABA in cultured cells were similar to those in leaves (Fig. 4); however, the rise in free and bound ABA levels during acclimation was observed sooner in cultured cells than in leaves. These

Fig. 4. Changes in free and bound endogenous ABA levels in suspension-cultured cells of grapes during heat acclimation. The cultured cells of 'Venus' (A) and 'Veeblanc' (B) were acclimated at 36°C, and free (●) and bound (○) ABA levels were determined in cells during acclimation.

results suggest that ABA accumulation in leaves and cultured grape cells was associated with high-temperature acclimation. Similar observations have been noted in many plant species exposed to temperature stress (Hiron and Wright, 1973; Rikin et al., 1976). A close association between ABA accumulation and peak cold tolerance was observed in potato (*Solanum tuberosum* L.) leaves (Chen et al., 1983). Although we observed rapid ABA accumulation in cultured cells during acclimation, the maximum levels of free and bound ABA in cultured cells were lower than those in leaves.

In leaves and cultured cells, there was a rapid increase of free and bound ABA in response to acclimation treatment. In leaves, levels of free and bound ABA increased $>$ 50% over the control within 1 h of acclimation. In cultured cells, free and bound ABA levels rose by $>$ 100% over the control during the first hour of acclimation. This increase accounts for $>$ 74% of the free ABA accumulation in cells occurring during acclimation. The sharp increase in ABA levels occurred well before leaves and cultured cells reached the maximum heat tolerance during acclimation. Nonetheless, peak ABA accumulation during heat acclimation coincided with peak heat tolerance, as with cold tolerance. However, ABA's response in relation to cold tolerance was observed after a few days of acclimation at low temperature and corresponded to peak cold tolerance (Chen et al., 1983).

Heat tolerance increased significantly (F test, $P < 0.05$) within 24 h of ABA application at 7.6 or 9.5 μM (Fig. 5). Cold tolerance has increased similarly in plants in response to exogenous ABA (Bomman and Jansson, 1980; Chen and Gusta, 1983; Rikin et al., 1979). ABA treatment has been shown to substitute for acclimation at low temperatures (Chen and

Fig. 5. Heat tolerance in cultured grape cells in response to exogenous ABA application. The cultured cells of 'Venus' (A) and 'Veeblanc' (B) were treated with ABA for 24 h, and lethal exposure time was determined based on the electrolyte leakage test.

Gusta, 1983; Chen et al., 1983). In many cases, the increase in cold tolerance from ABA application was greater than that from acclimation at low temperatures (Chen and Gusta, 1983). However, our results show that the increase in heat tolerance from exogenous ABA application was smaller due to acclimation at 36C. The increase in lethal exposure time for cultured cells from exogenous ABA applications ranged from 15 to 23 min. This diminished response to exogenous ABA application compared to acclimation at 36C suggests that ABA accumulation in cells is only one of many factors associated with the process of heat acclimation in grapes.

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