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Response of Tomato Plants to Stressful Temperatures[1]

INCREASE IN ABSCISIC ACID CONCENTRATIONS

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ABSTRACT

To investigate the abscisic acid (ABA) production of tomato (Mill.) plants in response to diurnal stressful temperatures, five-week old seedlings were exposed to day/night temperatures of 10/5, 15/10, 25/15, 35/25, or 45/35 C. The daylength was 16 hours with a light intensity of approximately 400 microeinsteins per meter per second. Plant tops were sampled at 12, 24, 48, and 72 hours. Free, alkaline-hydrolyzable (conjugated), and total ABA quantities were measured using standard gas chromatographic techniques. All temperature regimes significantly increased both free and conjugated ABA levels over concentrations in control plants (25/15 C). The highest ABA levels were observed in plants exposed to the coolest temperature of 10/5 C. Since normal water potentials were obtained in plants of all treatments, the observed ABA response was not due to temperature-induced water stress. Therefore, temperature stress, like several other environmental stresses, induces the plant to produce high levels of ABA. Because of the similar involvement of ABA in temperature-induced and other environmental stresses, ABA may be a common mediator for all plant stresses.

ABA is a naturally occurring compound of major importance in regulating plant growth and development. It has been implicated in a variety of physiological processes (1, 11, 13, 15) and is found in elevated levels under several stressful conditions (3, 4, 7, 8, 16, 18, 23).

The term "thermoperiodism" describes plants that grow better with variations in their day/night temperature regimes (24). For such plants, a constant temperature could be a stress. For example, tomato plants exhibit a profound reduction in growth at constant temperature (11). An alternating temperature regime is not only required for optimal vegetative growth but for other physiological processes like fruit set.

When tomato plants were exposed to constant stressful temperatures, an increase in their ABA levels was observed (2). The results did not indicate whether the increase was due to the stressful temperatures *per se* or to the fact that the plants were exposed to constant temperature. Here, an attempt was made to separate these two effects, namely, to determine whether tomato plants would respond in terms of ABA production when exposed to a stressful but a more natural diurnal temperature regime.

MATERIALS AND METHODS

Seeds of tomato (*Lycopersicon esculentum* Mill.) cv. Venus were sown in vermiculite[3] and germinated at 25 C. They then were transferred to a growth chamber set at 25 C day/15 C night. The photoperiod was 16 h (06:00–22:00 h), and the light intensity was approximately 400 μE m^{-2} s^{-1}. The seedlings were watered with one-quarter-strength Hoagland solution for the first week. When the plants were about 5.1 cm high, they were thinned to two plants/pot and, thereafter, were watered with full-strength Hoagland solution.

When the plants were 5 weeks old, they were exposed to day/night temperatures of 10/5, 15/10, 25/15, 35/25, or 45/35 C. The light period was maintained at the same duration and intensity. To minimize water stress from high temperature-induced dehydration, pots of every treatment were transferred to plastic trays containing full-strength Hoagland solution, and the solution was absorbed through holes in the bottom of the pots. The plants were allowed to absorb water for 2 h at 25 C before exposure to the experimental treatments. The leaf water potential of three plants from the 10/5, 25/15, and 45/35 C treatments was measured with a pressure bomb at regular intervals during the first 24 h.

Samples for ABA determination were harvested at 0, 12, 24, 48, and 72 h. Zero sampling time (09:00 h) was 3 h after the lights came on; therefore, all samples, except those at 12 h, were harvested 3 h after the lights came on. The 12-h samples were taken 1 h before the end of the light period (21:00 h). At each harvest, three single plant replicates (exclusive of the root system) were immediately frozen on dry ice and stored in a freezer at −18 C until analyzed. A representative sample (1–3 g) of each frozen and crushed plant was weighed, homogenized in ice-cold 90% methanol (10 ml/g fresh tissue), and filtered. ABA was analyzed according to the method described by Seeley and Powell (21). The alkaline-hydrolyzable ABA (conjugated ABA) was determined by adjusting the pH of the remaining aqueous phase to 11.0 with KOH, heating it at 60 C for 45 min, and re-extracting with methylene chloride. Acidic fractions were derivatized by ethereal diazomethane. ABA was quantified with a Tracor 222 Gas Chromatograph equipped with a Ni^{63} electron capture detector. Column packing was 3% OV-25 on Gas Chrom Q (100 to 200 mesh support). Purified N_2 at a flow rate of 80 ml/min was used as the carrier gas. The temperature of the injection port, column oven, and detector were isothermally maintained at 250, 225, and 295 C, respectively. Authentic (±)-ABA and racemic ABA were used as standards. Sample ABA peaks were identified by comparison with the retention time of authentic ABA.

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RESULTS

Any experiment dealing with temperature stress may be confounded by a temperature-induced change in the water status of the plant which would also affect ABA levels. The water potential of the plants in the experiment here was monitored during the first 24 h of the experiment (Table I). Since there was no difference in water potential of plants under different treatments during the initial 24-h period, no further measurements were made thereafter.

The lowest free ABA concentration was observed in control plants subjected to the optimal temperature of 25/15 C (Fig. 1). With the exception of the 12-h sampling, these values were significantly lower than the free ABA content of plants exposed to other temperatures. At 12 h, the free ABA contents of plants at 25/15 C or 15/10 C were not different.

After 12 h treatment, plants exposed to 10/5, 35/25, or 45/35 C exhibited a 1.5-fold increase in free ABA content as compared to the concentration at zero sampling time (t_0) but, at 15/10 C, it required 24 h for the plants to reach their highest free ABA levels. Free ABA concentrations of plants of all treatments showed a decreasing trend after 24 h exposure to different temperatures. However, plants subjected to the most stressful temperatures of 45/35 and 10/5 C showed a sharp and significant increase in their free ABA content at the 72-h sampling time. Although there was a decreasing trend in free ABA contents of plants subjected to 35/25 and 15/10 C throughout the experiment, the concentrations never returned to the initial values.

Results of the effect of temperature on conjugated ABA are presented in Figure 2. As with free ABA, lowest conjugated ABA levels were observed at 25/15 C. Conjugated ABA levels in plants subjected to 15/10 and 10/5 C continued to increase during the course of the experiment, the maximum being a 2-fold increase at 72 h. Plants exposed to 45/35 and 35/25 exhibited increased conjugated ABA levels during the first 12 h, a decrease back to initial levels at 24 h, and an increase during the latter part of the experiment. Plants subjected to 10/5, 15/10, or 45/35 C produced the highest levels of conjugated ABA. Regardless of sampling time, the plants followed a general pattern of lowest conjugated ABA at the optimal temperature and higher levels under stressful temperatures. After the initial increase at 45/35 C, the conjugated ABA levels declined to a minimum after 24 h and then gradually increased to near the 12-h level after 72 h. In contrast, the 35/25 C plants declined to a stable level after 48 h. At all times, conjugated ABA constituted the smaller fraction of the total ABA in tomato.

Minimal and constant total ABA levels were also observed in plants exposed to 25/15 C for all sampling times (Fig. 3). The highest total ABA levels were associated with the coolest temperature of 10/5 C for nearly all sampling times (but note the 12-h samples). At most times, total ABA levels of plants subjected to 45/35 C were higher than those exposed to 25/15 C, but lower than in plants growing at 10/5 C temperature regime. At 72 h,

Table I. *Water Potentials of Tomato Plants under Different Temperatures*
Values were obtained using a pressure bomb.

Time	Water Potentials at		
	45 C	25 C	10 C
h		*atm*	
0	−5.3	−5.3	−5.3
0.5	−6.5	−6.0	
2	−6	−6.5	
3	−5.5	−5.5	−6.5
6	−7	−7	
12	−7	−6	
24	−6	−7	−7

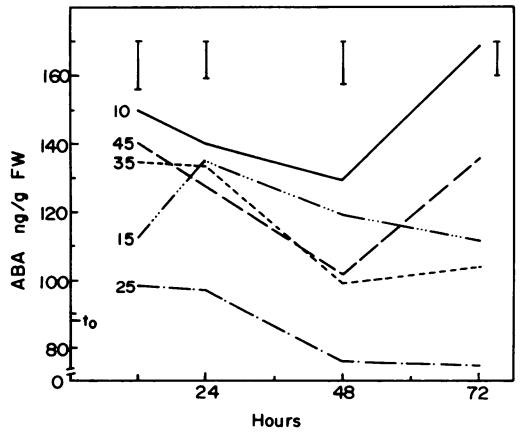

FIG. 1. Free ABA levels of tomato plants after 12, 24, 48, and 72 h exposure to different temperatures. t_0 is the zero sampling time. The vertical bar is the least significant difference at 5% level.

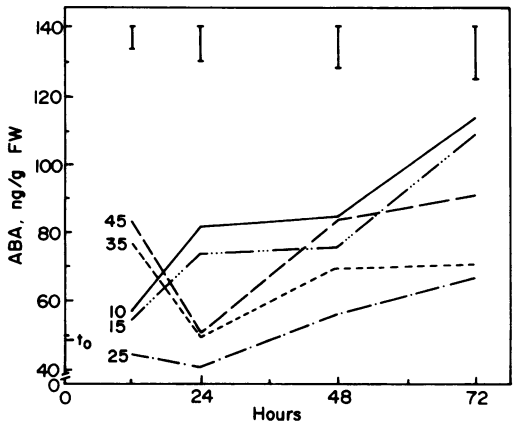

FIG. 2. Conjugated ABA levels of tomato plants after 12, 24, 48, and 72 h exposure to different temperatures. t_0 is the zero sampling time. The vertical bar is the least significant difference at 5% level.

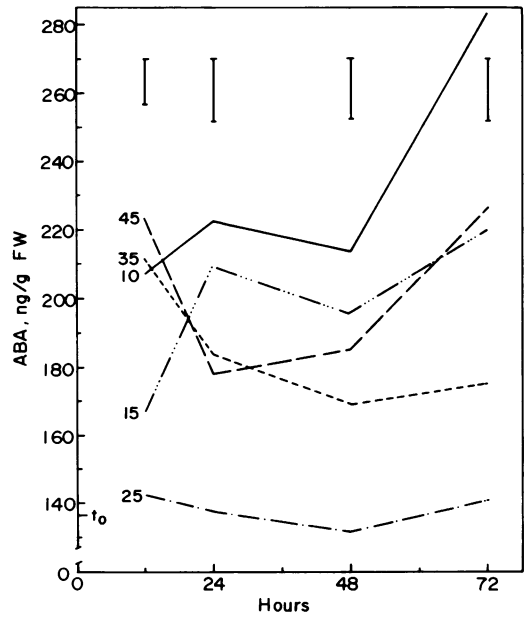

FIG. 3. Total ABA levels of tomato plants after 12, 24, 48, and 72 h exposure to different temperatures. t_0 is the zero sampling time. The vertical bar is the least significant difference at 5% level.

total ABA contents of plants exposed to 10/5, 15/10, 35/25, or 45/35 C temperature regimes were significantly higher than the initial total ABA present in the tissue. The maximum increase was observed after 72 h at the coolest temperature of 10/5 C and was double the initial level. Plants subjected to 10/5 or 15/10 C showed an increasing trend of their total ABA during the entire course of the experiment. There was a sharp increase in the total ABA of plants subjected to 45/35 or 35/25 C during the first 12 h.

A peak too small to be quantified, identified as trans-ABA, was observed in plants from all treatments. It occurred in both the free and conjugated forms. Milborrow (15) and Shaybany and Martin (22) have suggested that the presence of trans-ABA is due to photoisomerization of ABA in bright sunlight. The reason that a large ABA peak was not observed in the study here is probably because the tomato seedlings were grown in growth chambers under artificial lights, which not only have lower intensities than sunlight but also have a different spectral composition.

DISCUSSION

One mechanism for plants to dispose of harmful high levels of any compound is to metabolize and degrade the compound. Gillard and Walton (5) suggested that the most probable pathway for ABA metabolism is: ABA → 6′-hydroxymethyl ABA → phaseic acid → dihydrophaseic acid. Another method of inactivation would be to bind it to large molecules such as sugars (17). A binding mechanism for ABA definitely exists and operates throughout the plant (the presence of an alkaline hydrolyzable form of ABA attests to it). Although there is evidence for interconversion of ABA between free and conjugated forms, the results are only correlative and not conclusive (28) and several investigators have been unable to observe the proposed interconversion under various experimental conditions (1, 12, 14, 15).

Since the increase in free ABA observed at 10/5 and 45/35 C (between 12 and 48 h) was correlated to a simultaneous decrease in conjugated ABA, this initial increase may be the result of an interconversion from bound or conjugated ABA. However, the increase at 72 h seems to be the result of a *de novo* synthesis since there was no change in conjugated ABA.

Total ABA content of plants subjected to a diurnal temperature of 10/5, 15/10, 35/25, or 45/35 C were significantly higher than those of plants grown at the optimal diurnal temperature (25/15 C). This not only supports *de novo* synthesis of ABA but also suggests that although a binding and/or metabolic mechanism is involved, it is operating at a slower rate than the rates of synthesis. The presence of a binding mechanism is evident from the increased conjugated ABA levels under low and high temperature treatments (Fig. 2). The decline of temperature-induced elevated ABA levels to levels close to initial values further supports the involvement of a metabolic mechanism.

In most plant tissues, conjugated ABA concentrations are much lower than free ABA and, therefore, could not account for a significant change in the free ABA concentrations. Milborrow and Robinson (14), working with avocado plants subjected to wilting, concluded that the extra ABA present in wilted leaves arises by synthesis rather than by release from conjugated ABA. The data presented here suggest that, although both mechanisms are present, they may be operating in response to different environmental stimuli; however, the increase in ABA observed was apparently a result of synthesis.

Under acute stress conditions, ABA is known to increase up to 40-fold (11, 14). In the study here, the maximum increase observed was a 1.5- to 3-fold increase in the ABA level of plants exposed to 10/5 C; nevertheless, the increase under high or low temperature regimes is consistent and significant.

Similar increases in free ABA levels were observed in the leaves and crown tissue of wheat plants subjected to gradually decreasing diurnal temperature regimes (25). Therefore, it seems that temperature stress, *per se*, causes an increase in ABA levels of plants. These elevated ABA levels under temperature stress may have important physiological implications (18–20, 25, 26). Wightman (25) reported that, when wheat plants were subjected to decreasing diurnal temperature conditions over a 5-week period, ABA levels were increased to 3- and 10-fold, depending on the variety. Itai *et al.* (10) presented evidence that heat treatment increased the ABA levels in the xylem exudate of bean plants. In that experiment, ABA levels were higher 24 h after the plants had been "topped" and their roots dipped for 2 min in deionized H_2O of different temperatures. Roots exposed to 40 C had the same ABA levels as those subjected to 24 C. Only plants receiving 47.5 C treatment showed a 100% increase in their ABA levels (from 1.5–3.0 pg/ml) as compared to the controls (24 C). Although their report supports the hypothesis that ABA levels are modified by temperature, the results were obtained in a "physiologically disturbed" system, namely, after topping the plants. Here, evidence on the effect of temperature on ABA levels in an "intact" vegetative system is presented. Based on our data and the work of Wightman (25) and Itai *et al.* (10), it is conceivable that the plants respond to stressful temperatures by producing higher quantities of ABA. Although ABA production is very sensitive to the water status of the plant and although temperature changes may alter the internal water status, other factors beside temperature may be involved. No significant change in leaf water potential was observed here, and, therefore, it is concluded that the increased ABA was in response to temperature *per se* and not temperature-induced water stress.

The mechanism of action of ABA is clear in the case of water stress, namely, closure of stomates to prevent further water loss. ABA further improves the water balance of plants by increasing hydrolytic conductance (6, 19, 27). The mechanisms of action of ABA relative to other types of stresses, however, are not so clearly defined. Nevertheless, ABA levels have been elevated under several different environmental stresses, which seems to provide the plant with more "tolerance" (8, 9, 22). These findings suggest that ABA may be involved in a syndrome of responses, all of which may contribute to adaptation to environmental stress. In the case of temperature stress, ABA may enhance the plant's cold hardiness (20, 25) or may indirectly improve a plant's chilling sensitivity or heat tolerance by modifying its water balance. Support for this contention comes from the observation that chilling injuries are far less severe under conditions of high RH (19, 26, 27). The observation that the highest level of free, conjugated, or total ABA was observed in plants exposed to the coolest and the warmest temperatures respectively seems to be the result of the magnitude of the stress to the plant, lending itself to the speculation for a role of ABA in increasing the cold or heat tolerance of plants. The response of plants exposed to cold or heat stress conditions is similar to those subjected to drought stress. The full implication of these observations remains to be clarified.

Total ABA levels remained relatively stable throughout the experiment in plants held at the optimal temperature of 25/15 C. This fact illustrates the separation of the effects of constant stressful temperatures on ABA and those of stressful temperatures *per se* on ABA (Figs. 1–3). The main objective of this study, to prove or disprove this separation, was achieved; stressful temperatures, whether constant (2) or diurnal, caused an increase in ABA levels of tomato plants.

The change in ABA content caused by suboptimal or supraoptimal temperatures is similar to the involvement of ABA in other forms of stress. This supports a reasonable hypothesis: ABA is a common mediator for many different stresses.

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