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Determination of the Temperature Response Curves for Abscisic Acid and its Derivatives in Economically Important Horticultural Crops

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DETERMINATION OF THE TEMPERATURE RESPONSE CURVES FOR ABSCISIC ACID AND ITS DERIVATIVES IN ECONOMICALLY IMPORTANT HORTICULTURAL CROPS

by

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Jaleh Daie

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Determination of the Temperature Response Curves for Abscisic Acid and its Derivatives in Economically Important Horticultural Crops

by

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Department: Plant Science

Part I

Four-week old tomato (Lycopersicon esculentum Mill.) seedlings were exposed to different constant temperatures of 15, 25, 35, or 45 C. To determine the effect of temperature on Abscisic Acid (ABA) young and old leaves were harvested at 0, 6, 12, 24, or 48 h and free, hydrolyzable and total ABA were measured using gas liquid chromatographic methods. Temperature had a significant effect on free, hydrolyzable and total ABA in both young and old leaves. Time had a significant effect as a cubic function on all ABA measurements in old leaves but in young leaves a significant effect was observed only on the hydrolyzable ABA. Interactions between temperature and time were observed in both young and old

leaves. Young leaves had significantly higher amounts of free and hydrolyzable ABA than did old leaves. The total ABA levels were not significantly different in young and old leaves. Suboptimal temperatures increased ABA levels in the plants, in a manner similar to that of other kinds of stresses.

Part II

Five-week old tomato plants (Lycopersicon esculentum Mill.) were exposed to day-night temperatures of 10-5, 15-10, 25-15, 35-25, or 45-35 C. The day length was 16 h with a light intensity of approximately 400 μ E $m^{-2}s^{-1}$. Plant tops were sampled at 12, 24, 48, 68, or 72 h. Free, hydrolyzable and total absciscis acid (ABA) quantities were measured using standard gas chromatographic techniques. Stressful temperatures significantly increased both free and hydrolyzable ABA levels. The highest ABA levels were observed in plants grown at 10-5 C. Although time significantly affected ABA levels, its role needs more precise definition. Due to the similar involvements of ABA in temperature-induced and other stresses, ABA may be hypothesized to be a common mediator for different stresses.

Part III

Warm season crops: bean (Phaseolus vulgaris) CV. Burpee Stringless Green Pod; corn (Zea mays) CV. Golden Jubilee; Cantaloupe (Cucumis melo) CV. Hales Best; eggplant (Solunum melongena) CV. Ichiban; and okra (Hibiscus esculentus) CV. Dwarf Green and cool season crops: beet (Beta vulgaris) CV. Early wonder; lettuce (Lactuca sativa) CV. Great Lakes; cabbage (Brassica oleracea) CV. Savoy; radish (Raphanus sativus) CV. Scarlet Globe; and pea (Pisum sativum) CV. Little Marvel were exposed to 10, 25, or 40 C. After 24 h free and hydrolyzable ABA and DPA were measured in the plant tops using standard gas chromatographic methods. Warm season crops exhibited elevated levels of FABA, HABA and DPA under 10 C, compared to those at 25 or 40 C. Cool season crops showed similar FABA, HABA and DPA contents under all temperatures, except pea which had higher FABA and HABA levels at 40 C and beet which showed lower levels of HABA at 25 C as compared to 10 and 40 C. DPA existed at much higher concentrations than ABA in all plants of the two groups. It is concluded that while 10 C is stressful to warm season crops and thus the ABA and DPA increases, it is not so favorable to cool season crops. A temperature of 40 C would be stressful to both groups, but as long as water supply to the plant is not limiting the plants

would not actively produce ABA. Higher levels of DPA under stressful conditions would warrant consideration and taking into account the role of metabolism in regulation of total ABA in the plant.

(84 pages)

PART I

ABSCISIC ACID PRODUCTION IN *Lycopersicon esculentum* Mill: EFFECT OF CONSTANT TEMPERATURES

Introduction

The plant hormone abscisic acid (ABA) occurs in many plant parts in a wide spectrum of plant species. It is involved in dormancy, abscission, stomatal closure, senescence and a host of other physiological processes (Lindoo and Nooden 1978; Leopold and Kriedemann 1975; Milborrow 1974). ABA levels have been observed to increase under several different types of stresses; namely drought, salinity, disease, nitrogen deficiency, temperature and radiation, all of which impair the plant's growth and development (Daie et al. 1977 and 1979; Degani and Itai 1978; Dumbroff et al. 1977; Goldbach et al. 1975 and 1976; Irving 1969; Mizrahi et al. 1971 and 1972; Rakhimbaev et al. 1978; Simpson et al. 1972; and Wright and Hiron 1970).

Research on ABA can result in significant practical benefits. The observation that drought-tolerant species or varieties are capable of producing large amounts of ABA in comparison to non-tolerant ones, induced several groups to screen high ABA-producing agricultural crops in

attempts to breed new varieties capable of higher yields in dry regions (Jones 1978).

Cold acclimation, i.e., development of cold hardiness, is achieved in plants by being exposed to shorter days and lower temperatures. ABA is among several compounds found in high concentrations in plants as they attain cold hardiness (Holubowicz and Boe 1969; Levitt 1972). ABA is also known to increase the freezing resistance of several woody species (Irving and Lanphear 1978; Irving 1969; Rikin et al. 1975; Waldman et al. 1975). Freezing losses could be greatly reduced if it were possible to: (a) delay spring bloom of fruit trees for a week or two; (b) induce cold hardiness of many deciduous economic plants a week or two earlier than usual in the fall; (c) increase the level of cold resistance in mid-winter among warm season crops by only a few degrees; and (d) prolong the dormancy of plants that regularly begin to grow too early in the spring before all danger of frost is past (Weiser 1970). Elucidation of the role of ABA in relation to temperature would enhance our understanding in areas such as cold hardiness and frost resistance, and should also provide a greater understanding of plant growth and development under stressful environments.

The objectives of this study were: (a) to determine the effect of constant temperatures on ABA and its

conjugated derivative and (b) to compare the response of a stress on young leaves versus old leaves, as a function of time.

Materials and Methods

Tomato (Lycoperscion esculentum Mill.) plants were used because they are very responsive to temperature experiments. Seeds of "Venus" tomato were germinated in vermiculite and kept at room temperature. The plants were then transferred to a growth chamber set at 25 C day and 15 C night temperature and 16 hours of light of approximately 400 $\mu E\ m^{-2}s^{-1}$. The seedlings were watered with ¼-strength Hoagland's solution for the first week. When the plants were about 2 in. high, they were thinned to 2 plants per pot and watered with ½-strength Hoagland's solution.

Four-week old tomato plants were exposed to constant-temperature treatments of 15, 25, 35, or 45 C. The day length was again 16 h. To prevent water stress under high temperatures, all pots were transferred to plastic trays containing 3/4 strength Hoagland's solution. The solution was taken up by the plants through holes in the bottom of the containers. Water potential (Ψ_ω) of leaves was monitored during the first 24 h using a pressure bomb. Normal leaf Ψ_ω was observed.

Samples were harvested at 0, 6, 12, 24, or 48 h. At each harvest, 4 replicates of the 2 youngest leaves and the 2 oldest leaves were taken and immediately frozen with dry ice. Free (FABA), hydrolyzable (HABA), and total ABA (TABA) levels were determined. A representative sample of the frozen and crushed plant was weighed and homogenized in 75 ml of 90% chilled aqueous methanol. The homogenate was filtered and dried to an aqueous phase on a flash evaporator. The pH of the aqueous phase was changed to 8.5 and partitioned 3X with Methylene chloride ($MeCl_2$). The $MeCl_2$ was discarded, the pH of the aqueous phase changed to 3.0 and partitioned with $MeCl_2$. The $MeCl_2$ phase was dried, picked up in a few ml of solvent, dried under a stream of nitrogen, and methylated with diazomethane (Seeley et al. 1970). Hydrolyzable ABA was determined by changing the pH of the remaining aqueous phase to a value of 10.5 - 11.0. The sample was then heated in a waterbath at 60 C for 45 min. After being cooled, its pH was changed to 3.0, partitioned with $MeCl_2$ and methylated.

Methylated acidic fractions were analyzed using a Tracor 222 Gas Chromatograph equipped with a Ni^{63} electron capture detector. Column packing was 3% OV-17 on Gas Chrom Q with purified nitrogen at a flow rate of 80 ml/min as the carrier gas. The detector was purged with a flow rate of 10 ml/min. The temperature of the

injection port, column oven, and detector were isothermally maintained at 250, 225, 275 C, respectively. Authentic cis-trans ABA was used as a standard.

Results and Discussion

To estimate the effects of temperature and time in relation to level of ABA, a stepwise regression analysis was applied to each subset of experimental data. The relationship between the dependent variable (ABA) and independent variables (temperature and time) was estimated by a function consisting of constant, linear (L), quadratic (Q), and cubic (C) elements and interaction terms of independent variables. The statistical results are summarized in Tables 1 and 2. In the young leaves, temperature effect was significant in a linear relationship for FABA, HABA, and TABA and was significant for FABA and TABA as a quadratic function (Table 1). Time did not have any significant effect as quadratic and cubic functions but had a significant effect on HABA as a linear function. The interaction between temperature and time were significant for HABA, but were not considered in the model for TABA and FABA. The analysis of the old leaves data is presented in Table 2. Temperature had a significant effect on FABA, HABA, and TABA as a linear function and on FABA and TABA as quadratic and cubic functions. Interaction terms were significant

Table 1. Stepwise regression analysis of the effects of temperature and time on free, hydrolyzable and total ABA in young leaves of tomato

Subset	Entry Status			Significance at 5% level		
	F	H	T	F	H	T
time (L)	O	I	I	-	*	n.s.
temp (L)	I	I	I	*	*	*
$time^2$ (Q)	I	I	I	n.s.	n.s.	n.s.
$time^3$ (C)	O	I	I	-	n.s.	n.s.
$temp^2$ (Q)	I	O	I	*	-	*
$temp^3$ (C)	I	O	O	n.s.	-	-
time x temp (L)(L)	O	I	O	-	*	-
time x $temp^2$ (L)(Q)	I	I	O	n.s.	*	-
$time^2$ x temp (Q)(L)	O	I	O	-	n.s.	-
$time^2$ x $temp^2$ (Q)(Q)	I	I	O	n.s.	n.s.	-

F = Free ABA

H = Hydrolyzable ABA

T = Total ABA

I = In

O = Out

L = Linear

Q = Quadratic

C = Cubic

* = Significant at 5%

n.s. = Not significant

Table 2. Stepwise regression analysis of the effects of temperature and time on free, hydrolyzable and total ABA in old leaves of tomato

Subset	Entry Status			Significance at 5% and/or 1% level		
	F	H	T	F	H	T
time (L)	I	I	I	n.s.	n.s.	n.s.
temp (L)	I	I	I	*	*	*
$time^2$ (Q)	I	I	I	**	n.s.	**
$time^3$ (C)	I	I	I	**	*	**
$temp^2$ (Q)	I	I	I	*	n.s.	*
$temp^3$ (C)	I	I	I	*	n.s.	*
time x temp (L)(L)	O	O	I	-	-	*
time x $temp^2$ (L)(Q)	I	O	O	*	-	-
$time^2$ x temp (Q)(L)	I	O	O	n.s.	-	-
$time^2$ x $temp^2$ (Q)(Q)	I	O	I	n.s.	-	n.s.

F = Free ABA

H = Hydrolyzable ABA

T = Total ABA

I = In

O = Out

L = Linear

Q = Quadratic

C = Cubic

* = significant at 5%

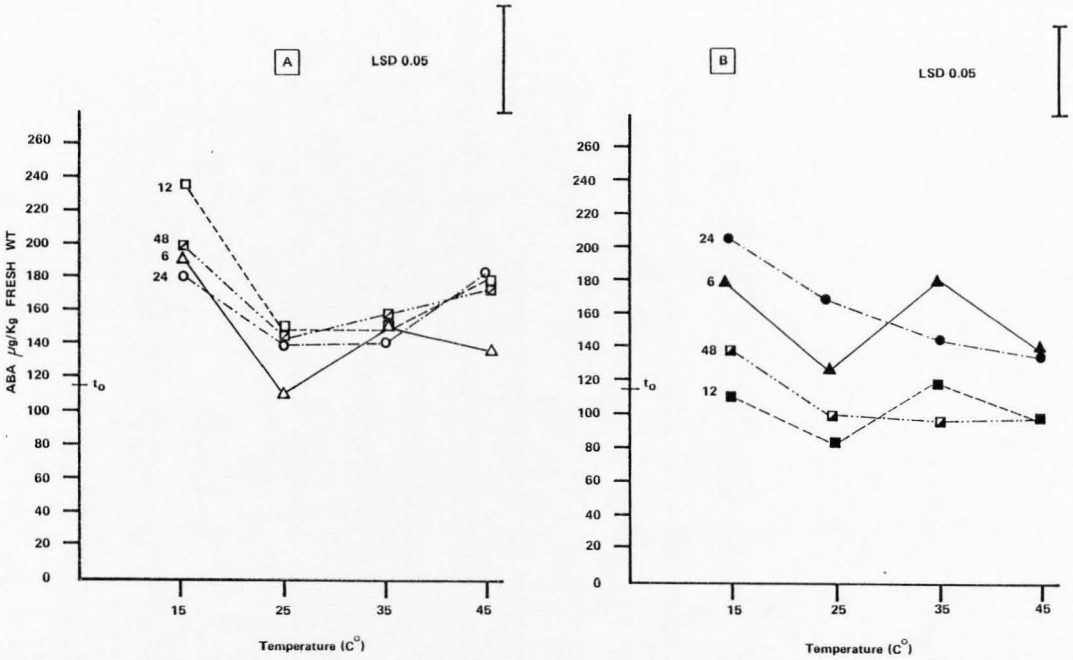
** = significant at 1%

n.s. = not significant

for FABA and TABA, but were not included in the model for HABA.

Temperature effects on ABA in young leaves. The highest amount of FABA was observed in plants grown under 15 C for all sampling times (Fig. 1A). The lowest amount of FABA was noted in plants grown under 25 C. However, at 12 and 24 h FABA was observed to be the same at both 25 and 35 C. After 6 h of incubation, FABA exhibited its highest level at the lowest temperature (15 C), was minimum at the optimal temperature (25 C) and increased again under the higher temperatures. Although the pattern of FABA production at 6 h was slightly different than other sampling times, the highest amount of FABA was still observed at 15 C while the 25 C treatment had the lowest amount of FABA.

Under all of the temperatures, the level of HABA remained almost constant during the first 6 h (Fig. 2A). At 12 h, there was a general increase in HABA with different temperatures, although the increase was more substantial at 25 and 35 C. After 24 h of incubation, HABA remained minimal under 25 C and maximal under suboptimal temperatures of 15 and 45 C. At the end of the 48-h period, HABA levels had declined close to near those recorded at 6 h, although plants exposed to stressful temperatures still exhibited higher amounts of HABA. Under all of


A
LSD 0.05
ABA µg/Kg FRESH WT
260
240
220
200
180
160
140
120
100
80
60
40
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t_o
12
48
6
24
15
25
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Temperature (C°)
B
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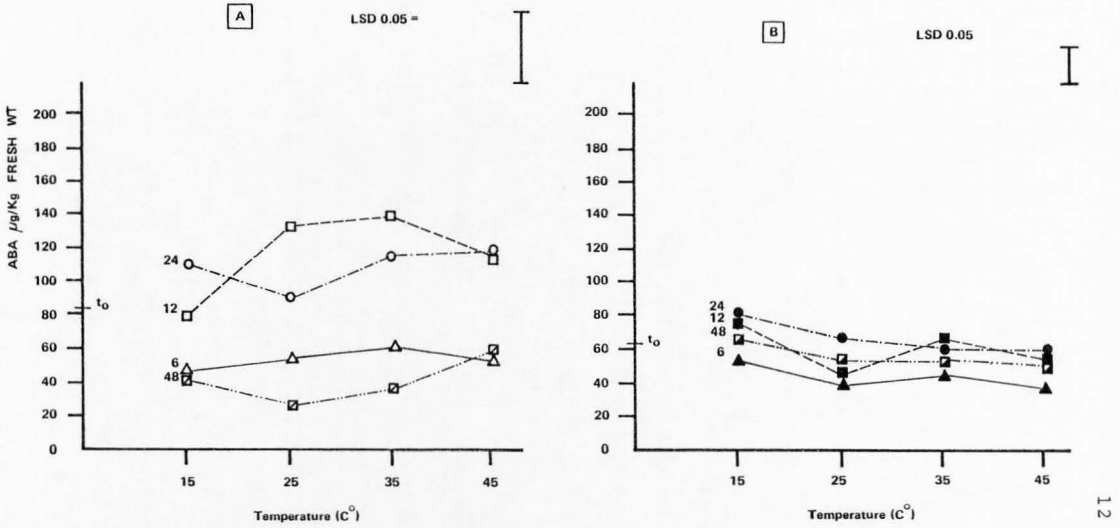
45
Temperature (C°)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Temperature (C°)
B
LSD 0.05
24
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48
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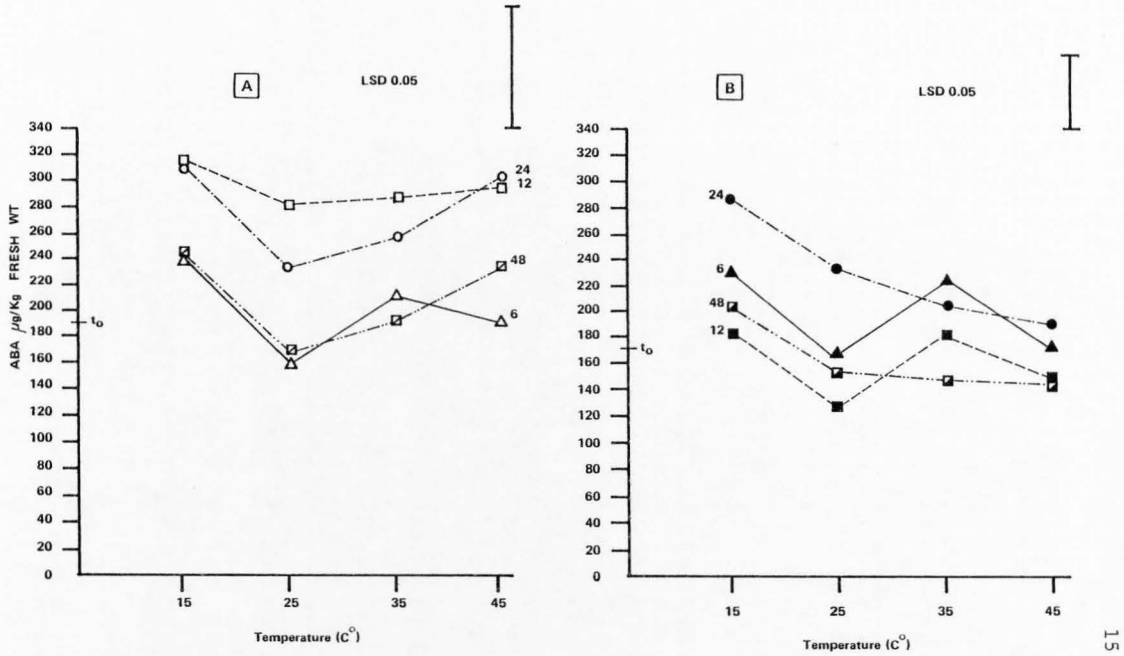
t_o
15
25
35
45
Temperature (C°)

the temperatures and/or sampling times, the level of HABA was lower than that of FABA (Fig. 1A and 2A).

Since the major component of the TABA was FABA, the changes in TABA levels with different temperatures closely resembled those of FABA, namely the highest levels under stressful temperatures and the lowest at the optimal temperature of 25 C (Fig. 3A).

Time effects on ABA in young leaves. There was an increase in FABA during the first 12 h of incubation at all temperatures (Fig. 1A). Twelve hours later (at 24 h), there was a significant decline in FABA of young leaves exposed to 15 C and a leveling-off in leaves of plants at 25, 35, and 45 C. The FABA levels of young leaves remained almost constant during the last 36 h of incubation (12-48 h), with higher levels of FABA measured in leaves of plants kept at stressful temperatures of 15 and 45 C.

There was a sharp and significant increase of HABA in young leaves during the first 12 h of treatment except when held at 15 C (Fig. 2A). The 48-h samples exhibited a sharp and significant decline in the HABA levels at all temperatures compared to HABA levels at 12 and 24 h. Plants exposed to the 15 or 45 C treatment had their highest levels of HABA after 48 h (Fig. 2A).


A
LSD 0.05
ABA µg/Kg FRESH WT
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
t_o
24
12
48
6
15
25
35
45
Temperature (C°)
B
LSD 0.05
24
6
48
12
t_o
15
25
35
45
Temperature (C°)

TABA levels followed a very similar pattern to those of FABA and HABA in young leaves (Fig. 3A). Compared to t_o values a sharp and significant increase occurred after 12 h under all temperatures, with the 15 C resulting in a higher level of TABA. TABA levels declined at 24 h and the decline continued to 48 h, when TABA levels reached values similar to those at 6 h. At 48 h, plants exposed to 15 or 45 C had higher amounts of TABA than did those grown at 25 or 35 C.

Temperature effects on ABA in old leaves. At 6 and 12 h sampling times, the lowest levels of FABA were observed in old leaves of plants grown under 25 C and the highest levels for these times were recorded in leaves of plants grown at 15 and 35 C (Fig. 1B). At 24 h, the level of FABA progressively decreased with increasing temperature. For the 48-h samples, the amount of FABA in old leaves of plants held under 25 C was significantly lower than in those of plants under 15 C, and remained constant as the temperature increased.

The levels of HABA in old leaves are shown in Fig. 2B. The only difference relative to the pattern of FABA observed in old leaves (Fig. 1B and 2B), is the magnitude of change, which was greatly reduced for HABA. As a result of this, the temperature response curves for TABA in old leaves (Fig. 3B) resembled that

of FABA (Fig. 1B). During the first 24 h, TABA contents of old leaves were significantly higher in plants held at 15 C than in plants grown at 25 C.

Time effects on ABA in old leaves. From time t_o, FABA increased sharply after 6 h and declined from 6 h to 12 h (Fig. 1B). Compared to 12 h, the 24 h samples showed a significant increase in FABA at 15 and 25 C. Subsequently, FABA decreased as temperature increased. At 48 h, FABA levels had declined, reaching levels close to those measured at 12 h. At the end of the 48 h incubation period, plants grown at 15 C had significantly more FABA than did plants at higher temperatures. The HABA levels of old leaves had slightly decreased after 6 h as compared to starting time but increased during the next 6 h of treatment (12 h). However, this increase was only significant for 15 and 35 C. Plants grown at 25 C showed an increase from 6 to 12 h to 24 h (Fig. 2B). At 24 h, the HABA levels remained steady and had declined slightly by 48 h. At 48 h, HABA levels were similar for all temperatures. Because the major component of TABA was FABA, the changes in TABA levels followed a very similar pattern to those for FABA. At every sampling time, the highest levels of TABA were obtained in plants grown at the coolest temperature (15 C) (Fig. 3B).

Response of young vs. old leaves. FABA, HABA and TABA in the young and old leaves of the tomato plants held at 4 temperatures and measured at 4 time intervals were averaged (Table 3). FABA and HABA contents within young leaves were significantly higher than in old leaves, but the difference between TABA levels was not significant. In both young and old leaves FABA was about two-thirds of the total.

The observation that young leaves have higher levels of ABA was also reported by Zeevart (1977). Sivakumaran et al. (1978) likewise reported that, under drought conditions, young seedlings responded more dramatically and that control young plants had higher levels of ABA. Zeevart (1977) suggested several possible explanations: (a) higher rates of ABA synthesis in younger tissue, (b) lower rates of metabolism in young tissue, (c) translocation from old to young tissues, and (d) a combination of these. It is, therefore, appropriate to take into account the age of the plant or tissue when attempting to ascertain any correlation between a particular plant process and changes in endogenous hormones.

ABA is inactivated in plants either by forming complexes with compounds such as sugars (Powell and Seeley 1974), or by being degraded via the phaseic acid pathway (Milborrow 1974). Wright (1975), reported a coincidence

Table 3. ABA content of young and old leaves of tomato

	ABA ug/Kg Fw				% of Total	
	Young	Old	Difference	% Difference	Young	Old
FABA	159.2[z]	129.8	29.4*	18.5	66.0	69.8
HABA	81.8	56.1	25.7*	31.4	34.0	30.2
TABA	241.0	185.9	55.1 n.s.	22.9	100.0	100.0

[z]Mean of 4 replicates of the 4 temperatures and 4 time intervals

*Significant at $P \leq 0.05$

of a rapid fall in free ABA and a rise in the level of hydrolyzable ABA in black-currant, which implies an inter-conversion of the two forms of ABA. The results in Fig. 1A and 2A indicate no evidence of a release of HABA into FABA form or vice-versa, except at 12 h, where the increase in HABA of young leaves coincided with the decrease of FABA at 25 and 35 C. The data are in agreement with results of Milborrow and Robinson (1973). Although the origin of the ABA required to maintain such elevated levels is unknown, it is unlikely that it is from the ABA-glucose ester, since the concentrations of ABA-glucose ester were lower in the nonstressed plants. Furthermore, Milborrow and Robinson (1973) reported that the conversion of exogenously applied ABA to its glucose ester was minimal in both stressed and nonstresed bean leaves. Similarly in the old leaves (Fig. 1B and 2B), no evidence indicated interconversion of FABA and HABA at any temperature or sampling time. The data (Fig. 3A) show an increase in TABA of young leaves under stressful temperatures. This increase could be the result of export from old leaves by a mechanism described by Zeevart (1977), who suggested that ABA and its metabolites were translocated in the phloem as well as being exported from mature leaves to other parts of the plant such as shoot tips, or it could involve a de novo synthesis of ABA. The

comparison of TABA in young leaves (Fig. 3A) and TABA in old leaves (Fig. 3B) did not totally support the idea of export from old leaves, although such an inference could be made from the data for plants grown at 35 or 45 C and sampling times of 24 or 48 h. The increase in TABA under stressful temperatures suggests the possibility of a de novo synthesis of ABA in the whole plant (cumulative of young and old leaves) or independently in young or old leaves.

ABA levels of both young and old leaves fluctuated during the 48 h of the study but reverted back close to the levels recorded at the beginning of the experiment. This indicates that, although temperature stress induced a relatively rapid increase of ABA, there probably existed another system by which ABA metabolism and/or binding was enhanced. The latter seems unlikely, because an increase in the bound form at the end of the 48 h was not evidenced.

Some reports in the literature regarding the effect of cold temperature on the breaking of dormancy and/or the disappearance of ABA in different parts of the plant suggest a temperature independent decline of ABA (Bonamy and Dennis 1977; Goldbach and Michael 1976; and Mielke and Dennis 1978). Rakhimbaev et al. (1978) in an attempt to clarify the role of phytohormones in the process of stratification, reported that the growth inhibitor

did not disappear but decreased slightly in tulip bulbs after cold treatment. They concluded that the growth and flowering of tulips was apparently the result of a favorable balance of phytohormones rather than a mere decrease in the levels of inhibitors.

There is only one report in the literature on investigations of the effects of high and low temperatures on ABA production from a stress point of view (Hellali and Kester 1979). The present data are sufficient to warrant assuming a role of temperature in ABA production in a vegetative system. Suboptimal temperatures affected ABA levels of the tomato plants in a manner similar to that of other kinds of stresses: namely, ABA production increased under stressful temperatures.

Tomato is a warm season crop and would grow even under temperatures in the upper 40s. Because of this, lower temperatures would be expected to cause more stress to the plant. The results of this study showed that in most cases, the amount of ABA was indeed highest at 15 C. ABA levels were high in plants grown at high temperatures, but not as high as in those under the cool temperature of 15 C, possibly reflecting the magnitude of the stress to the plant.

Although the mechanism of action of ABA is not clearly established, it can be speculated that high ABA levels could offer the plants an extra means for

survival when optimal conditions are not present in the environment. This assumption has been made by other investigators (Hellali and Kester 1979; Shaybany and Martin 1977). Working on bud failure of almonds and waterlogged walnut seedlings, respectively, they proposed that "resistant" plants react to the stress by producing larger quantities of ABA, than do "susceptible" varieties. The plant survives the adverse condition through this capability.

PART II

ABSCISIC ACID PRODUCTION IN Lycopersican esculentum MILL: EFFECT OF DIURNAL TEMPERATURE REGIMES

Introduction

The term "thermoperiodism" describes the conditions under which a plant would grow better with variations in its day-night temperature regime (Went, 1948). For such plants, a constant temperature could be a stress. Tomato plants are among those that exhibit a profound response when grown at a constant temperature (Leopold et al. 1975). The alternate temperature regime is not only required for optimal vegetative growth but for other physiological processes like fruit set.

The plant hormone abscisic acid (ABA) is a naturally occurring compound of major importance in regulating growth and development. It has been implicated in several physiological processes (Leopold and Kriedemann 1975; Lindoo and Nooden 1978; Milborrow 1974) and is found in elevated levels under several stressful conditions (Daie et al. 1979; Degani and Itai 1978; Dumbroff et al. 1977; Goldbach et al. 1975 and 1976; Irving 1969; Mizrahi et al. 1971 and 1972; Rakhimbaev et al. 1978; Simpson and

Saunders 1972; Wright and Hiron 1970). Increased ABA levels in leaves of tomato plants grown under stressful constant temperatures have been reported (Part I).

The objective of the present investigation was to determine the effects of a diurnal day-night temperature regime on ABA and its conjugated derivative as a function of time in tomato plants.

Material and Methods

Seeds of "Venus" tomato were sown in vermiculite and germinated at 25 C. They were then transferred to a growth chamber set for a 25 C day and a 15 C night temperature with 16 h of light at approximately 400 μ E $m^{-2}s^{-1}$. The seedlings were watered with 1/4-strength Hoagland's solution for the first week. When the plants were about 2 in. high, they were thinned to 2 plants per pot and then watered with full-strength Hoagland's solution.

Five-week old tomato plants 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 same duration and intensity. To prevent water stress from high temperature-induced dehydration, pots of every treatment were transferred to plastic trays containing full-strength Hoagland's solution and the solution was taken up through the holes in the bottom of the pots.

Using pressure bomb, normal leaf water potentials (Ψ_w) were observed for plants at all temperature treatments. Samples were harvested at 12, 24, 48, and 72 h (when lights were on) and at 68 h (7 hr after the lights were turned off). At every harvest 3 replicates of the whole plant (exclusive of the root system) were immediately frozen on dry ice and stored in a freezer until analyzed. A representative sample of each frozen and crushed plant was weighed and homogenized in 75 ml of ice-cold, 90% aqueous methanol and filtered. Free, hydrolyzable and total ABA (FABA, HABA, and TABA) were determined using the method previously described (Part I). Methylated acidic fractions were analyzed using a Tracor 222 Gas Chromatography equipped with a Ni^{63} electron capture detector. Column packing was 3% OV-25 on Gas Chrom Q, 100-120 mesh support. Purified nitrogen at a flow rate of 80 ml/min was used as the carrier gas. The detector was purged with a flow rate of 10 ml/min. The temperature of the injection port, column oven, and detector were isothermally maintained at 250, 225, and 295 C, respectively. Authentic cis trans and racemic ABA were used as standards.

Results and Discussion

A stepwise regression analysis was used to estimate the effects of temperature and time in relation to ABA

levels. The function consisted of linear, quadratic, cubic and interaction terms for both temperature and time (Table 4). Time had a significant effect on FABA as a linear, quadratic, and cubic relationship and was also significant for TABA in a linear and cubic relationship. Effects of time or temperature on HABA were not considered in the model except as a quadratic function of time, which was significant. The interaction term of linear x linear was significant for FABA and those of quadratic x linear and quadratic x quadratic were significant for both HABA and TABA. The other combinations were either not included in the model or were not significant.

Temperature Effect. In samples taken at 12, 24, 48, or 72 h, the lowest FABA contents were observed in plants grown at the optimum temperature of 25-15 C (Fig. 4A). With the exception of the 24 h sample, these values were significantly lower than FABA levels of plants exposed to 10-5 C. At 68 h (dark), the FABA levels of plants at 25-15 C were also significantly lower than those under 10-5 C and remained relatively constant as the temperature increased. In plants held under 45-35 C, FABA levels were higher than in those at 25-15 C; being significantly higher at 12 and 72 h.

Table 4. Stepwise regression analysis of the effects of temperature and time on free, hydrolyzable and total ABA in tomato

Subset	Entry Status			Significance at 5% and/or 1% level		
	F	H	T	F	H	T
Time(L)	I	O	I	**	--	*
Temp (L)	I	O	O	**	--	--
$Time^2$(Q)	I	I	I	**	**	n.s.
$Time^3$(C)	I	O	I	**	--	*
$Temp^2$(Q)	O	O	I	--	--	**
$Temp^3$(C)	I	O	I	**	--	**
Time x Temp(L)(L)	I	O	O	*	--	--
Time x $Temp^2$(L)(Q)	O	O	I	--	--	n.s.
$Time^2$ x Temp(Q)(L)	O	I	I	--	**	**
$Time^2$ x $Temp^2$(Q)(Q)	O	I	I	--	**	**

F = Free ABA

H = Hydrolyzable ABA

T = Total ABA

I = In

O = Out

L = Linear

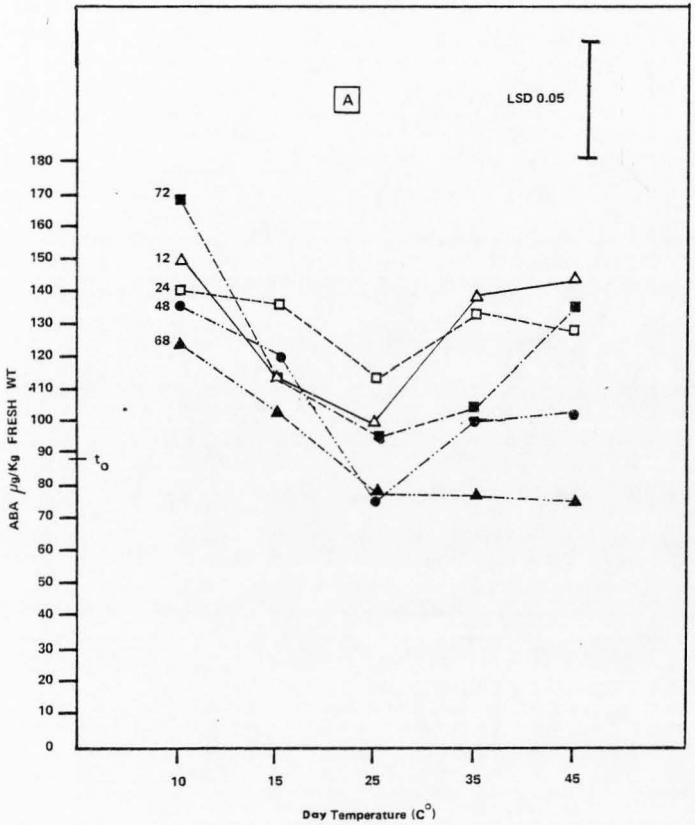
Q = Quadratic

C = Cubic

** = Significant at 1%

* = Significant at 5%

n.s. = Not significant

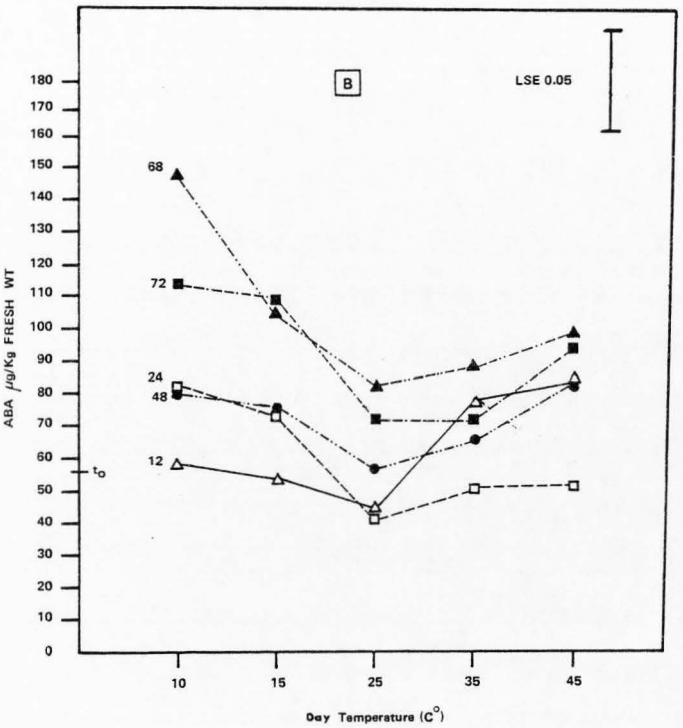

A
LSD 0.05
72
12
24
48
68
t_o
ABA µg/Kg FRESH WT
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10
15
25
35
45
Day Temperature (C°)

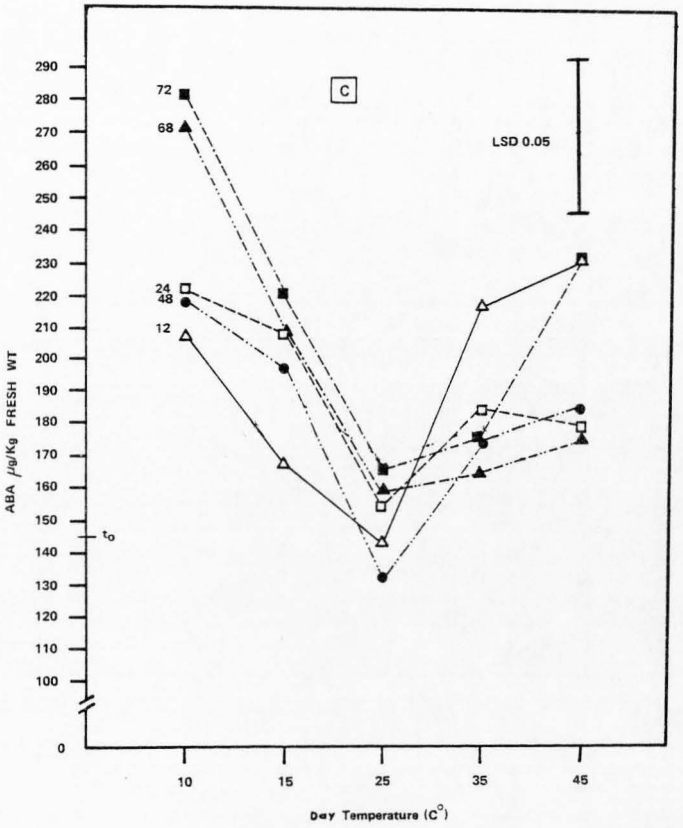
With the exception of the 68-h samples, all sampling times showed higher ABA levels at 45-35 C than at 25-15 C.

Results of the effect of temperature on HABA are presented in Fig. 4B. Minimal HABA levels were observed in the 25-15 C samples taken at 12, 24, 48, 68, or 72 h. At 12, 48, 68, or 72 h, HABA levels were higher under the 45-35 C regime as compared to those sampling times and plants at 25-15 C. However, only the 12 h samples showed a significant increase. At the 24 h sampling, the HABA levels remained relatively constant in plants at temperatures of 25-15, 35-25, or 45-35 C. Regardless of sampling time, the plants followed a general pattern of lowest HABA at the optimal temperature and higher levels under suboptimal temperatures.

Minimal TABA levels were also observed in plants growing at 25-15 C for all sampling times (Fig. 4C). The highest TABA levels were associated with the coolest temperature of 10-5 C for all sampling times, but the 12-h samples showed almost equal levels of TABA under the lowest and highest temperatures.

TABA levels of samples harvested at 24, 48, 68, or 72 h were higher in 45-35 C grown plants than those kept at 25-15 C, but lower than in plants growing at 10-5 C temperature regime. With the exception of the


B
LSE 0.05
68
72
24
48
12
t_o
ABA µg/Kg FRESH WT
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10
15
25
35
45
Day Temperature (C°)


C
LSD 0.05
72
68
24
48
12
ABA µg/Kg FRESH WT
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
0
10
15
25
35
45
Day Temperature (C°)

12 h sampling time, the highest TABA levels were associated with the coolest temperature of 10-5 C.

Time effect. After 12 h of incubation, the FABA levels of plants grown under diurnal temperatures of 10-5, 35-25, or 45-35 C were significantly higher as compared to levels found in those grown under 25-15 C or the initial value (t_o) (Fig. 4A). However, for plants exposed to 15-10 C, it required 24 h for the FABA levels to reach significantly higher levels than those measured at t_o. Throughout the experiment, FABA levels remained at their lowest in plants grown at 25-15 C and generally remained highest in plants subjected to 10-5, 15-10, or 45-35 C. At the dark-period sampling of 68 h, plants under 25-15, 35-25, or 45-35 C had amounts of FABA comparable to their initial values, while plants grown at 10-5 and 15-10 C had substantially higher amounts of FABA. After 72 h, FABA levels in plants grown under 10-5 or 45-35 C, remained significantly higher than their initial values or in those at 25-15 C and were comparable to the levels observed at 12 h.

HABA levels of plants growing at 25-15 C were also the lowest measured during the entire time of the experiment (Fig. 4B). Plants subjected to 10-5, 15-10, or 45-35 C temperatures exhibited the highest levels of HABA. After 72 h, HABA levels in plants grown under

25-15 or 35-25 C were not significantly different from the initial values, while those exposed to 10-5, 15-10, or 45-35 C were.

TABA levels were at their lowest in plants grown at 25-15 C and comparable to initial values throughout the experiment (Fig. 4C). They were highest in plants grown at 10-5 C. At 72 h, TABA levels in plants growing at 25-15 or 35-25 C were not different from their initial quantities, but were significantly higher in those exposed to 10-5, 15-10, or 45-35 C. Similar results were observed for FABA and HABA. At 68 h, TABA levels were not significantly different for plants grown at 25-15, 35-25, or 45-35 C. However, they were significantly higher in plants growing at 10-5 or 15-10 C.

One way for plants to dispose of harmful high levels of any compound is to bind it to large molecules such as sugars and thus inactivate it (Powell et al 1974). Although a binding mechanism definitely exists and operates throughout the plant (the presence of hydrolyzable ABA form attests to it), yet it may not be a prime way of disposing of ABA for the plant. Several investigators did not observe any interconversion of the two forms of ABA under different experimental conditions (Bonamy et al. 1977; Mielke et al. 1978; Milborrow et al. 1973). The present data support the conclusion of Milborrow

and Robinson (1973) that the increase in free ABA was due to de novo synthesis rather than to a release from a bound form since an increase in the bound form was also observed and any interconversion of FABA to HABA or vice versa was not evident (Fig. 4A, 4B, and 4C). The lack of an interconversion mechanism could not be ruled out, however, because there are reports to that effect (Wright 1975). A plausible explanation would be that, although the mechanism exists, it may not function in all plants or under all situations.

As previously reported (Part I of the dissertation), HABA constituted the smaller fraction of TABA in tomato. However, at 68 h, the HABA levels were higher than those of FABA (Fig. 4A and 4B). Also, at 68 h, when a generally decreasing trend was observed for FABA (Fig. 4A), the trend for HABA was upward (Fig. 4B): suggesting the possibility of rapid binding along with a rapid synthesis rate. Since the 68-h sample was taken during a dark period one could speculate that the ABA binding mechanism is more active in the dark. At 72 h (light period), the FABA levels were rising again and HABA levels were declining to levels similar to those at 48 h. TABA contents of plants grown under a diurnal temperature of 10-5, 15-10, or 45-35 C were higher than those of plants grown at the optimum diurnal temperature (25-15 C)

(Fig. 4C). This not only further supports the assumed de novo synthesis of ABA, but also suggests that, although a binding and/or metabolic mechanism is involved, it is operating at a much lower rate than the rates of synthesis. Furthermore, the presence of a binding mechanism is evident from the increased HABA levels under low temperature (Fig. 4B). The decline of temperature-induced elevated ABA levels to initial values further supports the involvement of a metabolic mechanism.

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 decreasing the root resistance to water efflux (Glinka 1971; Rikin and Richmond 1976). The mechanisms of action of ABA relative to other types of stresses, however, are not clearly defined. Nevertheless, ABA levels have been elevated in several cases of environmental stresses, which seems to render the plant more "tolerance" (Hellali and Kester 1979; Shaybany and Martin 1977). These findings suggest that ABA may be involved in a syndrome of responses, all of which may contribute to adaptation to environmental stress. In case of temperature stress, ABA may indirectly modify a plant's chilling sensitivity and heat tolerance by improving its water balance. Support for this contention

comes from the observation that chilling injuries are far less severe under conditions of high relative humidity (Rikin and Richmond 1976; Wright 1974; Wright and Simon 1973). The full implication of these observations remains to be clarified.

A peak too small to be quantitized that was identified as trans, trans ABA (t, t ABA) was observed in plants under all treatments. It occurred in both the free and conjugated form. Shaybany and Martin (1977) and Milborrow (1970) suggested that the presence of t, t ABA is due to photoisomerization of cis trans ABA in bright sunlight. The reason that a large t,t ABA peak was not observed in this study is probably because the tomato seedlings were grown in growth chambers under artificial lights, which had a different spectrum and intensity than sunlight.

Itai et al. (1973) presented evidence that heat treatment increased the ABA levels in the xylem exudate of bean plants. The levels were higher 24 h after the plants had been "topped" and their roots dipped for 2 minutes in deionized water 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 ABA levels (from 1.5 pg/ml to about 3.0) as compared to the controls (24 C). Although

their report supports the hypothesis that ABA levels are mediated by temperature, nevertheless the results were obtained in a "physiologically disturbed" system, namely after topping the plants. This report presents evidence of an effect of temperature on ABA levels in "intact" vegetative systems.

The fact that ABA levels remained stable throughout the experiment in plants held at 25-15 C, illustrates the separation of the effects of constant stressful temperatures on ABA and those of stressful temperatures *per se* on ABA (Fig. 4A, 4B, and 4C). The main objective of this study, to prove or disprove this separation, was achieved. Stressful temperatures, whether constant or diurnal, increased ABA levels in tomato plants (Part I of the dissertation). Although time had a significant effect on ABA levels as a linear, quadratic or cubic function, its role relative to ABA production seems to deserve further investigation.

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

PART III

ABSCISIC ACID PRODUCTION IN WARM SEASON CROPS VS. COOL SEASON CROPS AND METABOLISM OF ABA

Introduction

Abscisic acid (ABA) is a naturally occurring plant hormone with a major role in plant growth and development. It has been implicated in several physiological processes (Addicot and Lyon 1969; Leopold and Kriedemann 1975; Lindoo and Nooden 1978; Milborrow 1974). Furthermore, it is associated with different kinds of stresses (Daie et al. 1977 and 1979; Degani and Itai 1978; Dumbroff et al. 1977; Goldbach et al. 1975; Mizrahi et al. 1971 and 1972; Rakhimbaev et al. 1978; Simpson and Saunders 1972; Wright and Hiron 1970).

ABA seems to be involved in regulation of plants response to subzero temperatures; namely it increases the plant's resistance to them (Irving 1969; Rikin et al. 1975). This involvement has been interpreted by several investigators as a device to increase the plant's tolerance to the suboptimal conditions.

One of the limitations for production of a large group of economically important agricultural crops is

the temperature under which a plant can grow, survive and produce. Two groups are distinguished among vegetable crops: warm season and cool season crops.

In Part I and Part II of this dissertation, it was reported that the highest levels of ABA were observed under a cool temperature (10 or 15 C) in tomato, which is a typical warm season crop. These elevated levels were attributed to the magnitude of the stress to the plant.

The objective of this study was to determine the response of a group of cool season crops vs. warm season crops in regard to their ABA and its metabolite production under suboptimal temperatures.

Materials and Methods

Five species from each group were selected. The warm season crops were: bean (Phaseolus vulgaris) CV. Burpee stringless Green Pod; corn (Zea mays) CV. Golden Jubilee; cantaloupe (Cucumis melo) CV. Hales Best; eggplant (Solanum melongena) CV. Ichiban; and okra (Hibiscus esculentus) CV. Dwarf Green. The cool season crops were beet (Beta vulgaris) CV. Early wonder; lettuce (Lactuca sativa) CV. Great Lakes; cabbage (Brassica oleracea) CV. Savoy; radish (Raphanus sativus) CV. Scarlet Globe and Pea (Pisum sativum) CV. Little Marvel. The seeds were germinated in vermiculite at

room temperature. They were then transferred to a glass-house with natural lighting and at 25 C±2. A 6-10-4 fertilizer was applied biweekly. Later, 6-week old plants were transferred to a growth chamber maintained at 25-15 C day-night temperature regime with 16 h of light of about 400 μ E $m^{-2}s^{-1}$ and were conditioned for 48 h. Temperature treatments consisted of constant 10, 25 or 40 C. The light duration was the same as the conditioning period. To prevent water stress due to heat-induced dehydrations the plastic pots which had holes in the bottom were transferred to trays containing a full nutrient solution. Normal water potentials were observed. After 24 h of exposing the plants to different temperatures, 3 replicates of each treatment were sampled. Samples of the whole plant were immediately killed on dry ice and kept in a freezer until analyzed. Gas chromatographic methods, described previously (Seeley et al. 1970), were used. The only modification was that the basic aqueous phase was partitioned with petroleum ether. This procedure effectively removed the lipids. Methylated acidic fractions were analyzed for free ABA (FABA) and hydrolyzable ABA (HABA) using a Tracor 222 Gas chromatograph equipped with a ^{63}Ni electron capture detector. Column packing was a 3% OV-25 on Gas chrom Q, 100-120 mesh support. Purified nitrogen at a flow rate of 80 ml/min was used as the carrier gas. The detector

was purged with a flow rate of 10 ml/min. The temperature of the injection port, column oven and detector were isothermally maintained at 250, 225 and 295 C respectively. Authentic cis,trans ABA (c,t-ABA) racemic ABA and Dihydrophaseic acid (DPA) were used as standards.

Results and Discussion

Warm season crops. Free and hydrolyzable ABA showed a sharp and significant increase in all plants grown at 10 C as compared to levels found in plants grown at 25 and 40 C (Table 5). FABA content of different species was within the narrow range of 5-16 ng/gr fresh weight at optimal temperature (25 C). The increased FABA content of different plants at 10 C however, covered a wider range; 17.2-41.8 ng/gr fresh weight. For eggplant and okra this amounted to a 1.5 to 5-fold increase in FABA as compared to the levels at 25 C. The FABA levels of plants grown at 40 C with the exception of bean were either higher or the same as those grown at 25 C. Although the differences were not large, they were still significant.

The range of HABA of different species grown at optimum temperature was from 5 to 29 ng/gr fresh weight. The rate of HABA increase at the cool temperature was about 2-fold for eggplant and about 8-fold for cantaloupe. All plants exhibited a significant increase of their

Table 5. Free and hydrolyzable ABA content of warm season crops as affected by temperature

		ABA ng/gr FW		
		Temperature °C		
	Crop	10	25	40
FABA	Bean*	31.7[a]	16.0[b]	13.4[c]
	Corn	26.8[a]	6.8[b]	9.8[b]
	Cantaloupe	17.4[a]	4.6[b]	7.9[c]
	Eggplant	17.2[a]	12.0[b]	12.5[b]
	Okra	41.8[a]	8.6[b]	11.6[c]
HABA	Bean	51.8[a]	28.8[b]	26.4[b]
	Corn	21.7[a]	5.8[b]	7.9[b]
	Cantaloupe	52.0[a]	5.2[b]	4.2[b]
	Eggplant	36.9[a]	16.9[b]	15.3[b]
	Okra	50.3[a]	6.9[b]	6.5[b]

*Values within a row followed by the same letter are not significantly different at $P \leq 0.05$. Each value is the average of 3 replications.

HABA under 10 C compared to those at 25 C. There was no significant difference in HABA content of plants grown under 25 C or 40 C. In many plants, including tomato, FABA constitutes the major component of Total ABA (TABA). This is not the rule, however, as bean, eggplant and cantaloupe contained much higher levels of HABA than of FABA. Since both FABA and HABA levels (thus TABA) were raised at 10 C, a de novo synthesis of ABA rather than a release from a bound form is proposed. Warm season crops grow best at higher temperatures, but their growth is impaired when the temperature reaches near zero and cannot survive freezing temperatures even for a short period of time. The results of this study are supportive of this observation. Although 10 C is not destructive to plants' growth and function, nevertheless, they responded by producing high levels of ABA, thus possibly increasing their chances of survival should even lower temperatures occur. The low temperature could be perceived by the plants as a signal for potentially lower and more damaging temperatures. When tomato plants were grown under 45 C they showed higher ABA levels than those at 25 C (Part I and II of the dissertation). Thus, for warm season crops, it may also be speculated that temperatures above 40 C may induce significant increases in ABA levels.

ABA is metabolized either to its conjugated

derivative, HABA, or degraded via the phaseic acid (PA) pathway to DPA (Milborrow 1974; Powell and Seeley 1974). Gillard and Walton (1976) has suggested that the most probable pathway for ABA metabolism was: ABA → 6' hydroxymethyl ABA → PA → DPA. Harrison and Walton (1975) reported that under water stress conditions the first major metabolite was PA, but after about 30 h DPA was the major metabolite. An attempt was made to correlate DPA to temperature (Table 6). With the exception of eggplant, DPA levels were highest in plants grown at 10 C. DPA levels of bean and okra decreased as the temperature increased. Eggplant exhibited no change in DPA at any temperature. Corn and cantaloupe showed the highest levels of DPA at 10 C and essentially equal amounts at 25 and 40 C. The increase in DPA levels along with those of ABA have been observed in other stress conditions such as wilting (Harrison and Walton 1975). They proposed that this was due to high rates of synthesis and metabolism which were probably going on at equal rates. The fact that DPA levels of unstressed plants in this study were lower suggests that there may be a rapid turnover of DPA in the absence of stress and that the increase in metabolism in stressed plants results from an increased substrate availability. It is also conceivable that either due to elevated ABA levels or

Table 6. DPA concentrations of warm season crops as affected by temperature

	DPA ng/gr FW		
	Temperature °C		
Crop	10	25	40
Bean*	276.2[a]	115.0[b]	86.2[c]
Okra	223.1[a]	84.6[b]	54.6[c]
Corn	269.7[a]	102.4[b]	120.9[b]
Cantaloupe	250.0[a]	96.2[b]	120.2[b]
Eggplant	86.5[a]	88.4[a]	85.3[a]

*Values within a row followed by the same letter are not significantly different at $P \leq 0.05$. Each value is the average of 3 replications.

the stress itself, the metabolic enzyme activities were increased which lead to the high rates of metabolism (Harrison and Walton 1975).

The present data are in agreement with findings of several investigators who found that plants contain much larger quantities (up to 100-fold in dry bean seeds) of DPA than ABA concentrations whether or not they are under stress conditions (Harrison and Walton 1975; Walton et al. 1973; Walton et al. 1976).

Cool season crops. Plants of this group are those that grow under a wider range of temperature. They survive freezing temperatures of early spring and they grow well into the hot days of summer. Results of the effect of temperature on FABA and HABA on five cool season crops are presented in Table 7. With the exception of peas, all plants exhibited similar levels of both FABA and HABA under all temperatures. Peas had higher levels of FABA and HABA at 40 C than those at 10 or 25 C. Beet had its lowest HABA content at 25 C. This kind of response may be due to the fact that, while 10 C is not stressful to these crops, 40 C may be marginal. If 40 C is, in fact, stressful, then the plants may react to this high temperature signal by producing ABA when water is a limiting factor. Under the experimental conditions, the soil was maintained saturated and thus

Table 7. Effect of temperature on free and hydrolyzable ABA in cool season crops

		ABA ng/gr FW		
		Temperature °C		
	Crop	10	25	40
FABA	Beet*	56.0[a]	36.4[a]	43.9[a]
	Cabbage	8.4[a]	9.8[a]	9.3[a]
	Lettuce	6.1[a]	4.7[a]	7.0[a]
	Pea	2.4[a]	3.3[a]	9.4[a]
	Radish	5.3[a]	3.8[a]	5.8[a]
HABA	Beet	94.0[a]	66.7[b]	108.4[a]
	Cabbage	40.2[a]	31.4[a]	29.5[a]
	Lettuce	2.7[a]	2.7[a]	3.2[a]
	Pea	1.8[a]	1.7[a]	6.9[b]
	Radish	5.4[a]	1.3[a]	3.2[a]

*Values within a row followed by the same letter are not significantly different at $P \leq 0.05$. Each value is the average of 3 replications.

the plants remained fully turgid at 40 C. Among the cool eason crops, beet exhibited more than 10-fold amounts of ABA than the other plants (both FABA and HABA). The rest of the plants exhibited comparable ABA concentrations. The FABA and HABA contents within beet, lettuce, radish, and pea were similar, whereas the cabbage had a much higher HABA level than FABA. The general observation is that bound ABA constitutes a smaller portion of the total ABA.

The DPA content of the cool season crops was not significantly different at any temperature (Table 8). As with FABA and HABA, beet exhibited the highest level of DPA, while other plants had very similar DPA contents. Based on these data and previous observations (Harrison et al. 1975; Walton et al. 1976) where ABA metabolism is increased under stressful conditions, it may be speculated that the ABA metabolizes into DPA at a faster rate once the temperature drop lower than 10 C or are elevated above 40 C.

Comparison of the two groups of plants in their response to the same temperatures, supports the hypothesis of ABA production under stressful temperatures. Although a significant increase of FABA was observed in cantaloupe and okra at 40 C, as compared to 25 C, this increase was less than 50%. The increase in FABA at 10 C for all warm season crops relative to FABA concentrations

Table 8. DPA concentration of cool season crops as affected by temperature

	DPA ng/gr FW		
	Temperature °C		
Crop	10	25	40
Beet*	243.1[a]	230.1[a]	231.0[a]
Cabbage	93.0[a]	87.7[a]	182.0[a]
Lettuce	86.0[a]	63.9[a]	69.5[a]
Pea	88.4[a]	74.9[a]	73.9[a]

*Values within a row followed by the same letter are not significantly different at $P \leq 0.05$. Each value is the average of 3 replications.

at 25 C was up to 5-fold. One could assume that this was a reflection of the magnitude of the stress to the plant, because 10 C is indeed suboptimal and would be an important signal for a warm season crop.

Trans,trans ABA (t,t-ABA) of both groups were quantified. Some plants had excessively high levels up to 15 times as much as c,t-ABA and some had comparable amounts of t,t-ABA to those of c,t-ABA. However, no correlation with temperature could be established. This would be expected because the isomerization of c,t-ABA to t,t-ABA is a light dependent process (Milborrow, 1970).

The involvement of ABA, as well as its derivatives; the bound ABA, PA and DPA, is well documented in a number of stresses. Therefore, it seems plausible to consider that in any research dealing with the regulation of total levels of ABA, the role of metabolism should not be overlooked.

CONCLUSIONS

1. Abscisic acid levels of tomato are mediated by temperature.

2. Stressful temperatures whether constant or diurnal result in increased abscisic acid levels of tomato.

3. Both free abscisic acid and its conjugated derivative are affected by temperature.

4. Young leaves of tomato exhibited higher concentrations of abscisic acid than old leaves.

5. The major component of abscisic acid in tomato was the free form of the hormone constituting about 2/3 of the total concentration.

6. The increase in abscisic acid levels appears to be due to a *de novo* synthesis rather than release from a bound form.

7. Warm season crops exhibited highest levels of abscisic acid when exposed to cool temperatures. Cool season crops had similar abscisic acid concentrations under all temperatures. This may be the reflection of the magnitude of the stress to the plant.

8. As the abscisic acid levels increased under stressful temperatures, its metabolism into dihydrophaseic acid was also enhanced.

9. Due to similar involvement of abscisic acid in several stresses, it seems reasonably to hypothesize that abscisic acid is a common mediator for many different stresses.

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APPENDICES

Appendix A

In order to determine if the duration of exposure to light had any effect on ABA levels, a supplementary experiment to Part I was carried out. Three-week old tomato seedlings grown in a glass house were exposed to the optimal temperature of 25 C and suboptimal temperatures of 10 and 45 C for a period of 91 h. The photoperiod was 16 h of light and 8 h of dark and the cycle was maintained throughout the experiment. Samples of the entire top portion of plants were taken at 2 different sampling times. One group was sampled 3 h after the lights went on in the morning and the second group was sampled 3 h after the lights went off. To minimize heat induced water deficit, plants of all treatments were transferred to trays containing nutrient solution which was taken up through the holes at the bottom of the pots. ABA levels were measured using methods described in Part I.

The result of the experiment are presented in Fig. 5A, 5B and 5C. While the suboptimal temperatures caused an increase in both free and hydrolyzable ABA, the effect did not seem to be related to the duration of time that the plant was exposed to light or darkness.

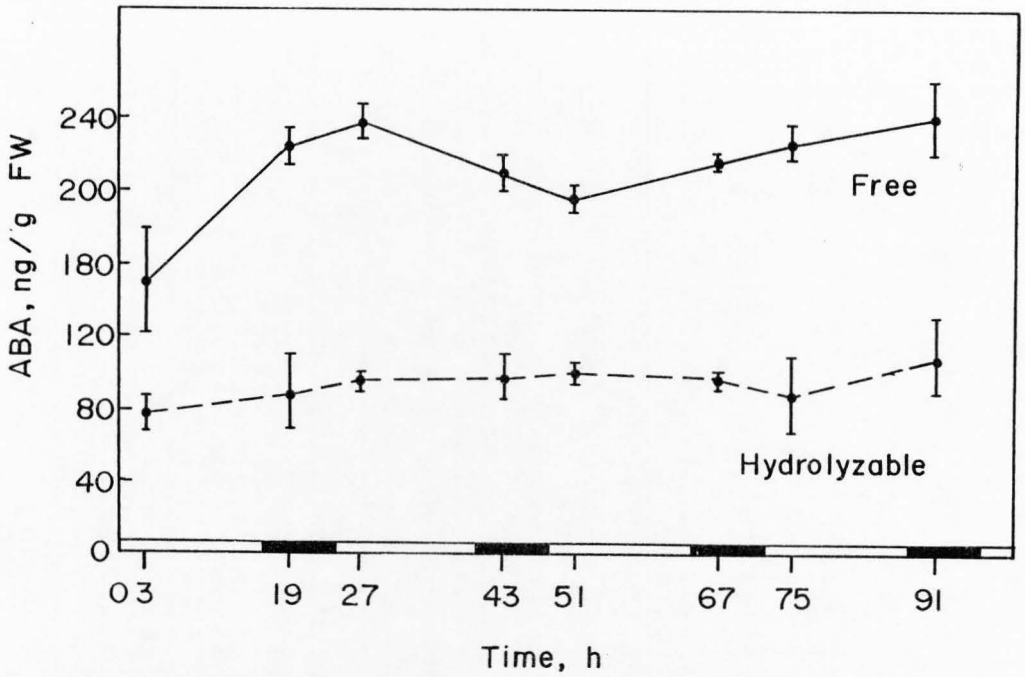

ABA, ng/g FW
240
200
180
120
80
40
0
Free
Hydrolyzable
0 3
19
27
43
51
67
75
91
Time, h

Fig. 5B. Free and hydrolyzable ABA concentration of tomato plants grown at 25 C. Samples were taken after 3 h of exposure to light (3, 27, 51, 75 h) or dark (19, 43, 67, 91 h).

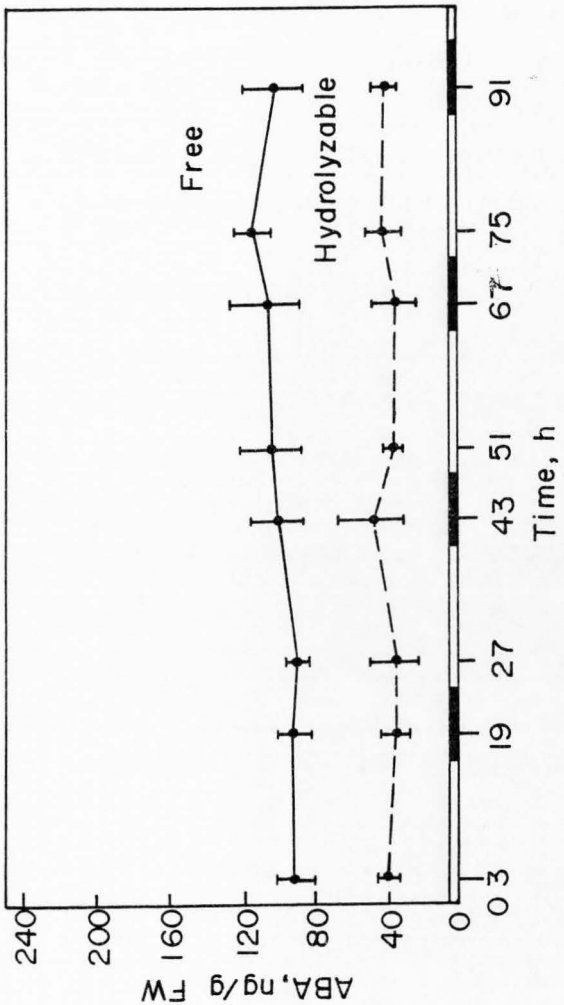
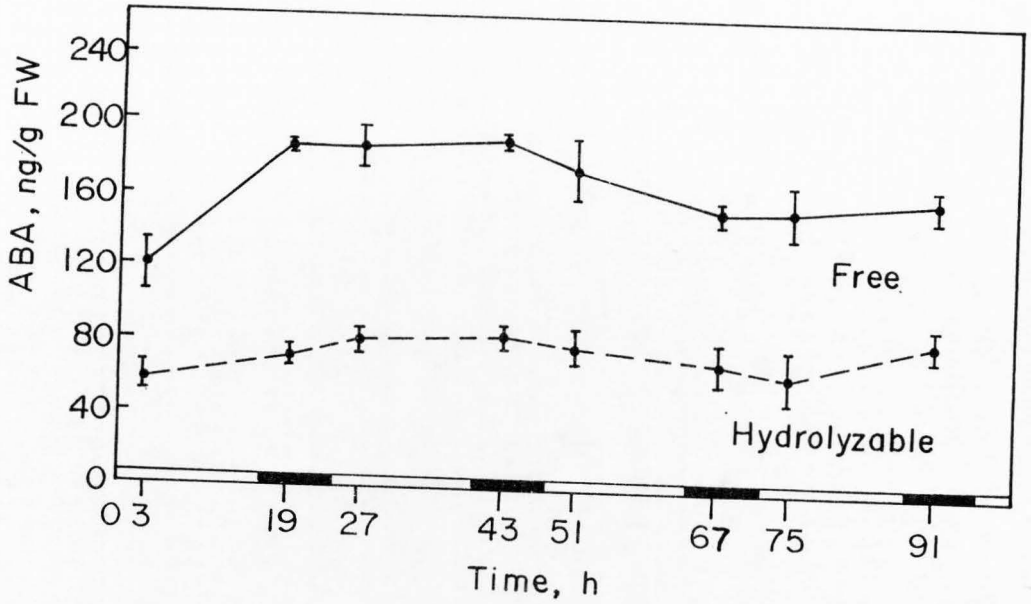

240
200
160
120
80
40
0
ABA, ng/g FW
0 3
19
27
43
51
67
75
91
Time, h
Free
Hydrolyzable

Fig. 5C. Free and hydrolyzable ABA concentration of tomato plants grown at 45 C. Samples were taken after 3 h of exposure to light (3, 27, 51, 75 h) or dark (19, 43, 67, 91 h).


ABA, ng/g FW
240
200
160
120
80
40
0
Free
Hydrolyzable
0 3
19
27
43
51
67
75
91
Time, h

Accordingly the adverse effect of low or high temperature overrides any role that light might have in determining the endogenous ABA concentration. Furthermore, the effect of light may be absent in tomato, since tomato is a day-neutral plant and does not respond to photoperiodism.

Appendix B

Table 9. Average values for free (F), hydrolyzable (H), and total (T) ABA during the 48 h of experiment in young and old leaves of tomato plants exposed to constant temperatures

Leaf Age	Temperature C°	ABA mg/gr FW		
		F	H	T
Young	15	201.8*	70.8	272.6
	25	134.0	75.5	209.5
	35	148.2	88.0	236.2
	45	143.5	87.0	230.5
Old	15	157.0	69.8	226.8
	25	117.2	50.5	167.7
	35	131.8	56.0	187.8
	45	113.5	49.3	162.8

*Each value is the average of 4 replicates of 4 sampling times of the experiment in Part I.

Table 10. Average values for free (F), hydrolyzable (H) and total (T) ABA in tomato plants exposed to diurnal temperatures.

Temperature C°	ABA mg/gr FW		
	F	H	T
10-5	143.6*	96.8	240.4
15-10	116.8	68.6	185.4
25-15	95.2	58.6	153.8
35-25	112.4	70.4	182.8
45-35	117.0	81.8	198.8

*Each value is the average of 3 replications of 5 sampling times of the experiment in Part II.

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