17. McMahon, D. 1975. Cycloheximide is not a specific inhibitor of protein synthesis *in vivo*. Plant Physiol. 55:815–821.
18. Mitsuhashi, M., H. Shibaoka, and M. Shimokoriyama. 1969. Morphological and physiological characterization of IAA-less-sensitive and IAA-sensitive phases in rooting of *Azukia* cuttings. Plant & Cell Physiol. 10:867–874.
19. Molnar, J. M. and L. J. La Croix. 1972. Studies of rooting of cuttings of *Hydrangea macrophylla:* DNA and protein changes. Can. J. Bot. 50:387–392.
20. Molnar, J. M. and L. J. La Croix. 1972. Studies of rooting of cuttings of *Hydrangea macrophylla:* enzyme changes. Can. J. Bot. 50:315–322.
21. Oppenoorth, J. M. 1979. Influence of cycloheximide and actinomycin D on initiation and early development of adventitious roots. Physiol. Plant. 47:134–138.
22. Torrey, J. G. 1965. Chemical factors limiting lateral root formation in isolated pea roots. Physiol. Plant. 9:370–388.
23. Watson, J. D. 1965. Molecular biology of the gene. W. A. Benjamin, New York.
24. Yarmolinsky, M. B. and G. L. de la Haba. 1959. Inhibition by puromycin of amino acid incorporation into protein. Proc. Nat. Acad. Sci. (U.S.A.) 45:1721–1729.

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Temperature-stress-induced Production of Abscisic Acid and Dihydrophaseic Acid in Warm- and Cool-season Crops[1]

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Abstract. **Abscisic acid (ABA) metabolism of 6-week-old seedlings of cool- and warm-season crops was determined after a 24-hr exposure to supra- and sub-optimal temperatures. Plants were grown at 25° C and then exposed to 10, 25, or 40°C. After a 24-hr exposure, free (FABA) and hydrolyzable (HABA) abscisic acid and dihydrophaseic acid (DPA) were measured in the plant tops by gas chromatography. Warm-season crops, exposed to 10°C exhibited elevated levels of FABA, HABA and DPA compared to those plants exposed to 25 or 40°C. Among cool-season crops, only peas had higher FABA and HABA levels at 40°C than at 10 or 25°C, while beets had lower levels of HABA at 25°C than at 10 or 40°C. DPA existed at much higher concentrations than FABA and HABA in all plants. The increases in ABA and DPA in warm-season crops exposed to 10°C are attributed to low temperature stress.**

Abscisic acid (ABA), a naturally occurring plant hormone, fills major roles in plant growth and development (1,10,11,12) that include an association with stress (3,4,5,7,14,15,20,25). The higher its ABA, the more resistance a plant will have to sub-zero temperatures (10,17,18). Several investigators believe that ABA can increase a plant's tolerance to any sub-optimal condition (9,10,17).

One limitation affecting the production of a large group of economically important agricultural corps is the temperature under which the plants can grow, survive, and produce. In tomato plants exposed to low or high temperatures, the highest levels of ABA were observed under 10°C (2). These elevated ABA levels were attributed to the magnitude of the temperature stress on the plant, since tomato is a warm-season crop.

The objective of this study was to determine whether concentrations of ABA and its metabolites in a group of cool- and warm-season crops were altered by exposure to supra- and sub-optimal temperatures.

Materials and Methods

Warm-season crops: bean *(Phaseolus vulgaris* L. cv. Burpee Stringless Green Pod); corn *(Zea mays L.* cv. Golden Jubilee); muskmelon *(Cucumis melo* L. cv. Hales Best); eggplant *(Solanum melongena* L. cv. Ichiban); and okra *(Hibiscus esculentus* L. cv. Dwarf Green) and cool-season crops: beet *(Beta vulgaris* L. cv. Early Wonder); lettuce *(Lactuca sativa* L. cv. Great Lakes); cabbage *(Brassica oleracea* L. cv. Savoy); radish *Raphanus sativus* L. cv. Scarlet Globe); and pea *(Pisum sativum* L. cv. Little Marvel) were sown in vermiculite and germinated in a glasshouse with natural lighting and maintained at 25±2°C. The seedlings were watered with 1/4-strength Hoagland solution for the first 2 weeks and with full-strength solution thereafter. When they were 6 weeks old they were transferred to a growth chamber with a day/night temperature of 25/15°C. The photoperiod was 16 hr (0600-2200) and the light intensity was approximately 400 μE $m^{-2}s^{-1}$. After a 48-hr pre-conditioning period in the growth chamber, the temperature was changed to constant 10,25, or 40°C.

To prevent water stress as a result of heat-induced dehydration, pots of all treatments were transferred to trays containing full-strength nutrient solution. Holes in the bottom of the pots allowed adequate uptake of solution. The light period was maintained at the same duration and intensity. The leaf water potential of plants was measured with a pressure bomb and did not differ with the temperature treatments. After 24-hr exposure to the treatment, single-plant samples from each chamber were harvested in triplicate. Top portions were harvested, frozen on dry ice and kept in a freezer at about – 20°C until analyzed.

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A representive sample (1–3 g) of each frozen and crushed plant was weighted and 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 (19). The alkaline-hydrolyzable ABA (HABA) was determined by adjusting the pH of the remaining aqueous phase to 11.0 heating at 60°C for 45 min and reextracting with methylene chloride. Acidic fractions were derived by etheral 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, 100-120 mesh support. Purified nitrogen at a flow rate of 80 ml/min was the carrier gas. The temperatures of the injection port, column oven, and detector were isothermally maintained at 250, 225 and 295°C, respectively. Dihydrophasic acid (DPA) was quantified in the acidic fraction containing the free ABA (FABA). Authentic (±)–ABA; racemic (±) – ABA, and DPA (received from Dr. D.C. Walton, State Univ. of N.Y., Syracuse, N.Y.) were used as standards. Sample peaks were identified by comparison with the retention time of the authentic compound. The first peak was ABA and then immediately t-ABA and finally DPA. The retention times were 2.25, 2.50, and 6.25 min, respectively.

Results and Discussion

In warm-season crops, FABA and HABA contents were significantly higher in all plants exposed to 10° C than in plants exposed to 25 or 40°C (Table 1). FABA contents of different species were within the narrow range of about 5–16 μ g/kg fresh weight at 25°C. At 10°C, however, FABA contents ranged from 17.2–41.8 μ g/kg fresh weight depending on the species. Eggplant showed a 1.5-fold increase while okra had a 5-fold increase in FABA as compared to their 25°C contents. At 40°C, FABA levels, except in bean plants, were either higher or the same in plants grown at 25°C.

The range of HABA of different warm-season species grown at 25°C ranged from 5 to 29 μ g/kg fresh weight. At 10°C, increases in HABA were 2- and 10-fold for eggplant and muskmelon, respectively. All plants exhibited a significant increase of HABA at 10°C compared to their levels at 25°C. There was no significant difference in HABA content of plants exposed to 25 or 40°C.

DPA levels of bean and okra decreased as the temperature increased (Table 1). Corn and muskmelon showed the highest levels of DPA at 10°C and had essentially the same amounts at 25 and 40°C. Except for eggplant, maximum DPA levels were observed in plants exposed to 10°C.

With the exception of peas, all cool-season plants exhibited similar levels of both FABA and HABA under all temperatures (Table 2). Peas had higher levels of FABA and HABA at 40°C than at 10 or 25°C. Beet plants contained the lowest HABA content, which was found in plants held at 25°C. Among the cool-season crops, beet plants exhibited up to 10-fold more FABA and up to 50-fold HABA than the other plants. The rest of the plant species had comparable ABA concentrations. Among treatments, the levels of FABA and HABA contents for lettuce, radish, and pea were similar, whereas the beet and cabbage had a much higher HABA than FABA levels.

The DPA contents of the cool-season crops did not differ significatly at any temperature (Table 2). As with FABA and HABA, beet plants contained more DPA than did the other plants, while the other plants had similar DPA concentrations.

Warm-season crops grow best at 25 to 30°C, and their growth is impaired at 10°C. Although this temperature does not destroy their growth and function, nevertheless, they produce high levels of ABA, perhaps as a way to increase their chances of survival

Table 1. Free (FABA) and hydrolyzable (HABA) ABA and dihydrophaseic acid (DPA) content of warm-season crops as affected by temperature.

Crop	Plant content (μg/kg fresh wt) 10°C	25°	40°
	FABA		
Bean	31.7a	16.0b	13.4c
Corn	26.8a	6.8b	9.8b
Eggplant	17.2a	12.0b	12.5b
Muskmelon	17.4a	4.6b	7.9c
Okra	41.8a	8.6b	11.6c
	HABA		
Bean	51.8a	28.8b	26.4b
Corn	21.7a	5.8b	7.9b
Eggplant	36.9a	16.9b	15.3b
Muskmelon	52.0a	5.2b	4.2b
Okra	50.3a	6.9b	6.5b
	DPA		
Bean	276.2a	115.0b	86.2c
Corn	269.7a	102.4b	120.9b
Eggplant	86.5a	88.4a	85.3a
Muskmelon	250.0a	84.6b	54.6c

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

Table 2. Effect of temperature on free (FABA), hydrolyzable (HABA) ABA, and dihydrophaseic acid (DPA) in cool-season crops.

Crop	Plant content (μg/kg fresh wt) 10°C	25°	40°
	FABA		
Beet	56.0a[z]	36.4a	43.9a
Cabbage	8.4a	9.8a	9.3a
Lettuce	6.1a	4.7a	7.0a
Pea	2.4a	3.3a	9.4b
Radish	5.3a	3.8a	5.8a
	HABA		
Beet	94.0a	66.7b	108.4a
Cabbage	40.2a	31.4a	39.5a
lettuce	2.7a	2.7a	3.2a
Pea	1.8a	1.7a	6.9b
Radish	5.4a	1.3a	3.2a
	DPA		
Bean	243.1a	230.1a	231.0a
Cabbage	93.0s	87.7a	182.0a
Lettuce	86.0a	63.9a	69.5a
Pea	88.4a	74.9a	73.9a

[z]Mean separation within rows by Duncan's multiple range test, 5% level.

should lower temperatures occur. The low temperature could have been perceived by the plants as signaling the onset of even lower and more damaging temperatures. The high ABA levels also could have resulted from chilling injury, since temperatures of 12°C or lower are known to cause chilling injury to these crops (10,17,18,21). Since tomato plants exposed to constant 45°C showed higher ABA levels than those at 25°C (2), temperatures above 40°C or a longer period of exposure to 40°C may induce significant increases in ABA levels in warm-season crops.

Cool-season crops typically can survive freezing temperatures of early spring and yet grow well ıinto the hot days of summer. Our comparisons of how the 2 groups of plants responded to the same temperatures supported the hypothesis that ABA is produced under stress. The results observed in cool-season crops may indicate that while 10°C is not stressful to these crops, 40°C is marginally stressful. If temperatures higher than 40°C are stressful, then the plants may react by producing ABA when water is a limiting factor. Under the experimental conditions, the soil moisture was maintained at an adequate level, so the plants remained fully turgid even at 40°C.

Although a significant increase of FABA was observed in muskmelon and okra at 40°C, as compared to plants held at 25°C, this increase was less than 50%. In contrast, all warm-season crops showed up to 5-fold increase in FABA at 10°C relative to their concentrations at 25°C. This could reflect the maginitude of the stress to the plant because 10°C is indeed suboptimal for a warm-season crop.

Since both FABA and HABA and consequently total ABA (TABA) and DPA increased in warm-season crops at 10° C, a *de novo* synthesis of ABA rather than a release from its bound form is proposed. In many plants, including tomato, FABA constitutes the major component of TABA. This is not universal, however, as bean, eggplant, and muskmelon contained much higher levels of HABA than of FABA. When trans-ABA (t-ABA) of both groups was quantified, some plants had up to 15 times as much t-ABA, while other plants had comparable amounts of t-ABA and ABA. Since no correlation with treatment temperatures could be established, it seems that the isomerization of ABA to t-ABA is totally light dependent, as suggested by Milborrow (13).

ABA is metabolized either to its conjugated derivative, HABA or degraded via the phaseic acid (PA) pathway to DPA (13,16). Gillard and Walton (6) suggested that the most probable pathway for ABA metabolism was: ABA → 6' hydroxymethyl ABA → PA → DPA. Harrison and Walton (8) reported that under water-stress conditions the first major metabolite was PA, but after about 30 hr, DPA was the major metabolite.

Increases in DPA as well as ABA levels have been observed in plants under water stress by Harrison and Walton (8). They attributed this to simultaneously high rates of synthesis and metabolism. DPA levels were lower in unstressed (than stressed) plants in this study, suggesting a rapid turnover of DPA in the absence of stress and that the enhanced metabolism of ABA in stressed plants results from an increased substrate availability. It is also conceivable that metabolic enzyme activities would increase due to either elevated ABA levels or the stress itself, and lead to the high rates of metabolism of ABA (8). Based on our data and previous observations (8,22,23,24) of ABA metabolism increasing under stressful conditions, it may be speculated that the ABA would be metabolized into DPA at a faster than normal rate once temperatures are lower than 10°C or exceed 40°C.

The present data are in agreement with findings of several other investigators who have reported that plants contain much larger quantities (up to 100-fold in dry been seeds) of DPA than ABA regardless of stress conditions (8,23,24).

Literature Cited

1. Addicott, F.T. and J. L. Lyon. 1969. Physiology of abscisic acid and substances. Annu. Rev. Plant Physiol. 20:139–164.
2. Daie, J. 1980. Determination of the temperature response curves of abscisic acid and its derivatives in economically important horticultural crops. PhD Thesis, Utah State Univ., Logan.
3. Daie, J., S.D. Seeley, and W. F. Campbell. 1979. Nitrogen deficiency influence on ABA in tomato. HortScience 14:261–262.
4. Degani, N. and C. Itai. 1978. The effect of radiation on growth and abscisic acid in wheat seedlings. Environ. Expt. Bot. 18:113–115.
5. Dumbroff, E. B., D. C. W. Brown, and J. E. Thompson. 1977. Effect of senescence on levels of free ABA and water potentials in cotyledons of bean. Bot. Gaz. 138:261–265.
6. Gillard, D. F and D. C. Walton. 1976. Abscisic acid metabolism by a cell-free preparation from *Echinocystics lobata* liquid endosperm. Plant Physiol. 58:790–795.
7. Goldbach, E., H. Goldbach, H. Wagner, and G. Michael. 1975. Influence of N-deficiency on the abscisic acid content of sunflower plants. Physiol. Planta. 34:138–140.
8. Harrison, M. A. and D. C. Walton. 1975. Abscisic acid metabolism in water-stressed bean leaves. Plant Physiol. 56:250–254.
9. Hellali, R. and D. E. Kester. 1979. High temperature induced bud–failure symptoms in vegetative buds of almond plants in growth chambers. J. Amer. Soc. Hort. Sci. 104:375–378.
10. Irving, R. M. 1969. Characterization and role of an endogenous inhibitor in the induction of cold hardiness in *Acer negundo*. Plant Physiol. 44:801–805.
11. Leopold, A. C. and P.E. Kriedemann. 1975. Plant growth and development, 2nd ed. McGraw-Hill, New York.
12. Lindoo, S.J. and L. D. Nooden. 1978. Correlation of cytokinins and abscisic acid with monocarpic senescense in soybeans. Plant Cell Physiol. 19:997–1006.
13. Milborrow, B. V. 1974. The chemistry and physiology of abscisic acid. Annu. Rev. Plant Physiol. 25:259–307.
14. Mizrahi, Y., A. Blumenfeld, S. Bittner, and A. E. Richmond. 1971. Abscisic and cytokinin contents of leaves in relation to salinity and relative humidity. Plant Physiol. 48:752–755.
15. Mizrahi, Y. and A. E. Richmond. 1972. Abscisic acid in relation to mineral deprivation. Plant Physiol. 50:667–670.
16. Powell, L. E. and S. D. Seeley. 1974. The metabolism of abscisic acid to a water soluble complex in apple. J. Amer. Soc. Hort. Sci. 99:439–441.
17. Rakhimbaev, I. R., G. A. Syrtanova, and V. F. Solomina. 1978. Effect of cold treatment on the level of biological activity of endogenous growth regulators in Tulip bulbs. Soviet Plant Physiol. 25:197–200.
18. Rikin, A., M. Waldman, A. E. Richmond, and A. Dovrat. 1975. Hormonal regulation of morphogenesis and cold resistance. I–Modifications by abscisic acid and gibberellic acid on alfalfa *(Medicago sativa* L.) seedlings. J. Expt. Bot. 26:175–183.
19. Seeley, S. D. and L. E. Powell. 1970. Electron capture gas chromatography for sensitive assay of abscisic acid. Anal. Biochem. 35:530–533.
20. Shaybany, B. and G. C. Martin. 1977. Abscisic acid identification and its grantification in leaves of *Juglans* seedling during water-logging. J. Amer. Soc. Hort. Sci. 102:300–302.
21. Shneyour, A., R. M. Smillie, and J. K. Raison. 1975. Biochemical and genetic basis for the temperature sensitivity of photosynthesis and growth in chilling sensitive plants. p. 349–355. In: Y.E. Nasyrou and Z. Sestak (eds.) Genetic aspects of photosynthesis. Junk, The Netherlands.
22. Simpson, G. M. and P. G. Saunders. 1972. Abscisic acid associated with wilting in dwarf and tall *Pisum sativum*. Planta 102:272–276.
23. Waiton, D. C., B. Corn, and J. Fey. 1973. The isolation of an abscisic acid metabolite, 4'-dihydrophaseic acid, from non-imbided *Phaseolus vulgaris* seed. Planta 112:87–90.
24. Walton, D. C.., M. A. Harrison, and P. Cote. 1976. The effects of water stress on abscisic acid levels and metabolism in roots of *Phaseolus vulgaris* L. and other plants. Planta 131:141–144.
25. Wright, S. T. C. and R. W. P. Hiron. 1970. The accumulation of abscisic acid in plants during wilting and under other stress conditions. p. 291–298. In: D. J. Carr (ed.) Plant growth substances. Springer-Verlag, Berlin.