Iran Agricultural Research 11:77-88 (1992)

Foliarly Applied Abscisic Acid increases Cold Hardiness and Carbohydrates in *Actinidia arguta*

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(Received August 8, 1992)

ABSTRACT

Abscisic acid was applied foliarly to *Actinidia arguta* Ananasnaja plants in a growth chamber under simulated winter conditions and in a shade-house under natural winter conditions, to determine the influence of exogenously applied abscisic acid on cold hardiness and soluble carbohydrates. Carbohydrate levels were measured in November, January, and March. Cold hardiness of stem sections, measured by controlled freezing followed by electrical conductivity tests, was significantly increased by abscisic acid treatment. Enhanced cold hardiness was accompanied by increased soluble carbohydrate levels (288%, 209%, and 127% that of controls in November, January and March, respectively). The two sugars most responsive to abscisic acid treatment were melibiose and stachyose.

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تحقیقات کشاورزی ایران
۱۱:۷۷-۸۸ (۱۳۷۱)

افزایش قندها و مقاومت به سرما در گیاه کی‌وی با هورمون اسید آبسایزیک

عنایت‌اله تفضلی و کالا بیل
به ترتیب دانشیار بخش باغبانی دانشکده کشاورزی دانشگاه شیراز و استاد دانشگاه A & M آلاباما.

چکیده

هورمون اسید آبسایزیک بصورت محلول‌پاشی در اتاقک رشد با شرایطی شبیه به شرایط زمستانی و نیز در هوای آزاد بر روی گیاه کی‌وی پاشیده شد تا اثرات آن بر روی قندهای محلول و مقاومت گیاه به سرما اندازه گیری شود. ساقه‌های گیاه در ماه‌های آبان، دی و اسفند تحت سرمای مصنوعی قرار گرفتند و سپس میزان عبور الکتریسیته از آنها اندازه گیری شد. ساقه‌هائی که تحت تیمار قرار گرفته بودند مقاومت بیشتری در مقابل عبور الکتریسیته از خود نشان دادند. افزایش مقاومت با افزایش میزان قندهای محلول (به ترتیب به میزان ۲۸۸، ۲۰۹ و ۱۲۷ درصد در ماه‌های آبان، دی و اسفند در مقایسه با کنترل) همراه بود. ملیبیوز و استاکیوز از جمله قندهائی بودند که بیشتر از بقیه تحت تاثیر هورمون قرار گرفتند.

INTRODUCTION

Abscisic acid (ABA) is known to play a role in chilling and freezing resistance in a number of plants (2,4,6) and its level increases during dormancy and cold hardiness (7,11). Nonstructural carbohydrates, particularly the soluble sugars, serve an important role in freezing resistance.

Apple and sycamore exhibit a marked increase in soluble sugars during hardening that correlates with improved freezing resistance (12). This study was undertaken to investigate the effect of foliarly applied abscisic acid on both cold hardiness and carbohydrates in stem sections of *Actinidia arguta*, a relative of kiwifruit, during a dormancy cycle.

MATERIALS AND METHODS

Plant Material and Experimental Conditions

Nine-month old rooted cuttings of *Actinidia arguta* Ananasnaja were planted in 20-cm diameter plastic pots using Fafard-mix #2 (Cassco, Montgomery, AL) and divided into 2 uniform groups of 24 each. One group was placed outside, in a shade-house with 30% shade on the campus of Alabama A&M University, Normal, AL, so that it could experience the natural progression of winter occurring during 1991-1992; and the other group was placed in a Rheem constant environment reach-in growth chamber (Puffer-Hubbard Environmental, Weaverville, NC). The growth chamber was adjusted to simulate outside weather conditions, using monthly means for the previous 27 years of the period from September to March. Environmental conditions in the growth chamber were as follows a) mean temperature of 23.5°, 17.5°, 12°, 8°, 7°, 8° and 11,5° C for the months of September to March, b) relative humidities (%) of 80, 79, 81, 78, 63, 72 and 56 and c) 14, 13, 13, 12, 11, 12 and 13 hr of daylength. Day and night temperatures were the same. Each group was then further subdivided into two groups of 12 plants for growth regulator treatment. Abscisic acid (10^{-4}M) was applied foliarly until runoff in mid-September one week after potting. A drop of Tween-80 was added as a surfactant.

Carbohydrate Determination

Samples were collected for carbohydrate analysis three times during the season in November, January and March. In November, plants were sampled when the leaves on the control plants had began to yellow and abscise. In January, all treated and untreated plants appeared fully dormant and showed no evidence of vegetative or reproductive growth. In March, samples were obtained when leaf buds on control plants had began to expand initiating growth. Stem samples were lyophilized, weighed, ground, homogenized in a blender, and then extracted for carbohydrates. Samples of each plant (1 g dry weight) were extracted with 75 ml of 50% ethanol in

a 100 ml volumetric flask, placed in a sonicator-water bath for one hr at 50° C, cooled to room temperature (26° C), and then brought to 100 ml volume. The extracts were then centrifuged for 10 min at 2000 rpm and the supernatant was used for sugar analysis. Aliquots of the sample (10 ml) were filtered through a Gelman 0.45-mm Acrodisc LC filter after which 1 ml was taken and spiked with 10 mg of lactose internal standard before being injected into a Perkin-Elmer high pressure liquid chromatograph (Norfolk, CT) equipped with a solvent programmer. Samples were carried through the amino-bonded carbohydrate column (Waters, Malborough, MA) by 75% acetonitrile at a flow rate of 2 ml min^{-1} and detected with a UV visible spectrophotometer set at 190 nm wave length. Sugar standards (1%) were made and 200 µL of each were injected into the HPLC. The retention times and concentrations were determined and used for calculating the quantities of various sugars in the same extracts using the following formula (15):

$$C_{(i)}=F \quad \frac{f_{(i)} \times A_{(i)}}{f_{(s)} \times A_{(s)}} \times \frac{W_{(s)}}{W}$$

Where $C_{(i)}$ = amount of sugar to be calculated, F = conversion factor, $f_{(i)}$ = response factor of the unknown, $A_{(i)}$ = area peak of the unknown, $f_{(s)}$ = response factor of the internal standard, $A_{(s)}$ = area peak of the internal standard, $W_{(s)}$ = amount of the internal standard, and W = the amount of the sample. All extracts were sampled for sucrose, glucose, fructose, arabinose, maltose, melibiose, raffinose and stachyose.

Evaluation of ,Cold Hardiness

Stem sections of 1-cm length were collected in early January and immediately used in controlled freezing tests, followed by evaluation of electrical conductivity to determine their cold hardiness (9). An LT-50 low temperature circulating bath (Neslab Instruments, Dublin, CA)containing absolute methanol and equipped with an

ETP-30 electronic temperature programmer was used to cool stem samples which had been placed in glass vials (1.5x4 cm) from temperatures of 2.5° C to - 20° C at a rate of 2.5° C per hr.

Stem samples were removed from the low temperature bath after being exposed to three test temperatures (-10, -15 and -20° C) for one hr. After equilibration for 1.5 hr at room temperature, 10 ml of distilled water were added to each tube containing the stem section. Samples were then held at 20° C for 24 hr with intermittent stirring. The resultant solutions were then read with a YSI Model 32 conductivity meter (Yellow Springs Instrument Co, Yellow Springs, , OH) to obtain an initial conductivity value. The samples were then boiled for 5 min brought up to the original volume (10 ml) with distilled water, and conductivity was measured after 24 hr of intermittent stirring. Percent electrolyte leakage (EL) at each temperature was defined as (9):

$$EL\ (\%) = (\text{Initial EC}/\text{Final EC}) \times 100$$

Where EC = electrical conductivity measured.

Statistical Analysis

All data were subjected to analysis of variance as a completely randomized design in a 2×2×3 factorial arrangement, with 4 replications. An analysis of variance was performed for main effects and interactions of hormone treatment, location, and time of sampling, and in the case of conductivity values also for the temperature, using SAS (18).

RESULTS AND DISCUSSION

Many plants develop resistance when they are stressed and such a common mechanism of adaptation may be hormone-regulated, particularly with ABA (1).

Endogenous ABA prevented chilling injury in some plants (16,17) and increased plant hardiness (3,4). Our data indicate that foliarly-applied abscisic acid effectively increased cold hardiness (Fig. 1). There was a very highly significant effect of ABA on the percentage of electrolyte leakage from the stems which had undergone controlled freezing. The amount of electrolyte leakage from stem segments that had been exposed to different temperatures in controlled freezing tests was proportional to the amount of cold damage experienced by the stem segments. There was a significant negative relationship between temperature and electrical conductivity (Fig. 1). There were also significant interactions ($P>0.01$) for temperature, hormone, and location. In the growth chamber, environmental conditions were simulated using mean values for the winter months. In the shade-house, plants were exposed to the full fluctuations of temperature that occur in a normal winter season in Huntsville, Alabama. The beneficial effects of the ABA foliar application were greater in plants grown in the shade-house than in the growth chamber. Calculating the equations of different curves showed that linear curves were best fitted for the relationship between stem electrolyte leakage and the temperature. The equations for different locations and treatments are shown on the top of Fig. 1.

Table 1 shows the amounts of various carbohydrates in the stem segments of the test plants. There were significantly greater amounts of soluble carbohydrates in ABA treated plants. In terms of percentage of the control, ABA treated plants had 288%, 209% and 127% of the total carbohydrates of control plants in November, January and March, respectively. Total carbohydrates were highest in November and declined over time. The sugars which were the most reponsive to the ABA treatment were the disaccharide melibiose and the tetrasaccharide stachyose.

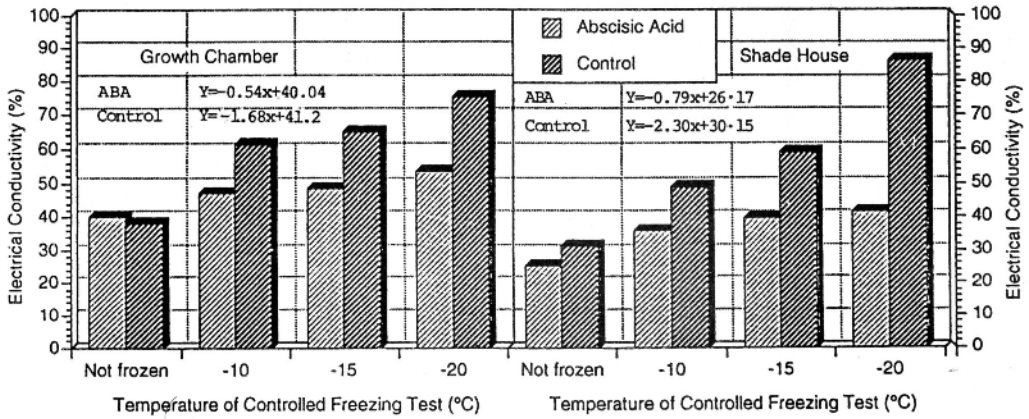

Fig. 1. Effects of foliarly-applied abscisic acid on cold hardiness of *Actinidia arguta* Ananasnaja plants grown in either growth chamber or in the shade-house. Stem segments were exposed to four temperatures in controlled freezing tests and cold damage is proportional to the amount of electrolyte leakage (%) measured. Significance of the ANOVA: Location (L)**, Temperature (T)**, Lx T**, Hormone (H)**, Lx H**, Tx H**, Lx Tx H**.

** Significant at %1 level of probability.

Foliarly applied ABA increased cold hardiness and corresponding soluble carbohydrate levels. A relationship has been reported for high soluble carbohydrates particularly glucose and fructose and freezing tolerance in *Actinidia chinensis* and *A. arguta* (10). They also reported that high amount of unsaturated to saturated fatty acids and high levels of ABA were correlated to freezing tolerance.

There are many reports linking endogenous ABA with high soluble carbohydrates in many physiological processes such as bud differentiation (20), fruit maturation (13,19) and cold tolerance (11), but there are few reports of the effects of foliarly applied ABA resulting on increasing soluble carbohydrates.

While studying the physiology of rice tungro virus disease, Mohanty and Sridhar (14) increased reducing sugars by applying ABA to senescing leaf blades. In a study of ABA's role in anthocyanin and sugar accumulation in skins of grape, Matsushima *et al.*(13) found that glucose, fructose and rhamnose increased more rapidly in skins of ABA-treated fruit bunches.

Exogenously applied ABA does not always mimic the effects of endogenous levels. Soybean seedlings transferred to low osmotic potential vermiculite had ABA levels 5-10 fold greater than controls but soluble sugar content did not change in seedlings that had been exogenously treated with ABA (5). In the present study, foliarly applied ABA increased cold hardiness and the increase was paralleled by a corresponding increase in soluble carbohydrates.

ACKNOWLEDGEMENTS

We thank Venu Oddiraju for his help in performing the statistical analysis, Shiraz University, Shiraz, Iran, for the sabbatical support of the first author.

Table 1. Influence of abscisic acid on carbohydrate content and levels (mg g^{-1} dry weight of stem) in *Actinidia arguta* treated exogenously with foliarly applied abscisic acid.

Treatment	Sucrose mg g^{-1}	Glucose mg g^{-1}	Fructos mg g^{-1}	Arabinose mg g^{-1}	Maltose mg g^{-1}	Melibiose mg g^{-1}	Raffinose mg g^{-1}	Stachyose mg g^{-1}	Total mg g^{-1}	% control
						November				
Control	0.88a*	0.85a	0.38b	0.57b	0.06a	0.75b	0.62a	0.00b	4.11b	100b
Abscisic acid	0.38b	0.84a	1.15a	4.18a	0.00b	3.82a	0.00b	1.48a	11.58a	288a
						January				
Control	0.91b	1.23a	0.36b	0.75b	0.00a	0.77b	0.41a	0.00a	4.43b	100b
Abscisic acid	2.10a	0.64a	0.68a	2.48a	0.01a	2.64a	0.58a	0.20a	9.27a	209a
						March				
Control	0.39a	0.48a	0.14b	0.46a	0.00a	0.00a	0.07a	0.23a	1.80b	100b
Abscisic acid	0.42a	0.67a	0.50a	0.07b	0.00a	0.01a	0.13a	0.47a	2.28a	127a

Means in each column for each month followed by the same letter are not significantly different at 1% levels of probability using t-test.

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