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Short Communication

Effects of pH and Ethephon on Betacyanin Leakage from Beet Root Discs

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MICHAEL S. REID, JACK L. PAUL, AND ROY E. YOUNG[1]
Department of Environmental Horticulture, University of California, Davis, California 95616

ABSTRACT

Betacyanin leakage from beet root discs was found to increase with decreasing pH of the incubation medium. Although 10 millimolar Ca^{2+} reduced pigment leakage at pH 3.5, it was ineffective at pH 2.3. Leakage was also stimulated by 100 micrograms per milliliter (2-chloroethyl)-phosphonic acid (pH 3.1), but when the solution was neutralized, this leakage was eliminated. Bubbling C_2H_4 through a neutral medium containing beet discs had no effect on pigment leakage; it appears that the effect of Ethephon solutions on this process is a function of their low pH.

It was recently shown that low concentrations of Ethephon (2-chloroethyl)phosphonic acid greatly stimulated the leakage of betacyanin from beet discs and that this effect could be reduced by adding Ca^{2+} and other polyvalent cations to the medium (5). C_2H_4, the plant growth regulator that is released by hydrolysis of Ethephon, causes a wide range of physiological changes in plant tissues. Many of these effects are related to plant senescence, and some of these have been associated with changes in membrane integrity (1). On the other hand, Ethephon is a strong acid; a concentration of 100 μg/ml in water has a pH of about 3. Low pH alone can have a deleterious effect on root tissue and causes leakage of cell constituents (3). Moreover, H^+-induced leakage can be greatly reduced by addition of relatively high concentrations of Ca^{2+} (3). In this paper, we examine the relative importance of H^+ and of C_2H_4 in the leakage of betacyanin from beet root discs.

MATERIALS AND METHODS

The experimental procedure used was similar to that described by Poovaiah (5) and Poovaiah and Leopold (4). Before treatment, beet discs, 1 cm in diameter and 1.5 mm thick, were washed in aerated, DI[2] for 2 h. The supernatant was poured off every 30 min and replaced with fresh water. Five discs were placed in 10 ml test solution in a 20-ml vial at 22 C, and leakage of betacyanin was assessed by measuring the O.D. of the test solution at 540 nm at the end of the treatment period. The discs were not shaken during treatment. Ethephon, (21% active ingredient) was supplied by Amchem Products Inc., Ambler, Pa. Each treatment was replicated three times, and the treatment period was 4 h. Ca loss from beet tissue was estimated by measuring the Ca content of the solutions at the end of the treatment period. The initial Ca content of the beet tissue was 1.3 μmol/g fresh weight. Ca content was measured by atomic absorption spectroscopy using $LaCl_3$ (2,000 μg La/ml) as the releasing agent. For determining the C_2H_4 production in various treatment solutions, beet discs were placed in 10 ml of solution in vials (20 ml) that were sealed with serum caps. The C_2H_4 content of the gas samples from the vials was determined by gas chromatography.

RESULTS

Effect of Ethephon and HCl on Betacyanin Leakage and Ca Loss. Solutions of 0, 50, 100, 250, and 1,000 μg/ml Ethephon and solutions adjusted to the same pH with HCl were tested. The initial pH values were 6.0 (DI water), 3.3, 3.0, 2.7, and 2.2, respectively. During the experiment, the pH values changed, and the final values were 5.5, 3.55, 3.4, 2.95, and 2.4 for both the Ethephon and HCl solutions. In Figure 1A pigment leakage is plotted as a function of the final pH. Leakage of betacyanin increased sharply below pH 4 in both the HCl and Ethephon solutions. At the lowest pH, all pigment had been completely removed from the discs in both treatments. There was no significant difference in the effect of pH on leakage between the two acids. The loss of Ca from the tissue is shown as a function of pH (Fig. 1B). Loss of Ca was affected by pH in the same way as the leakage of betacyanin. There was little difference between the

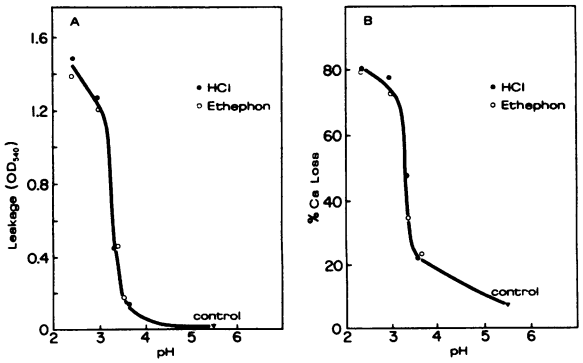

FIG. 1. Leakage of betacyanin and Ca^{2+} from beet discs treated with Ethephon or HCl over a range of pH. Beet discs were incubated for 4 h in varying concentrations of Ethephon or of HCl. The betacyanin content (A) and Ca^{2+} contents (B) of the solutions were then determined.

[1] Permanent address: Botany and Plant Science Department, University of California, Riverside, California 92521.

[2] Abbreviation: DI, deionized water.

acids.

Effect of Neutralized Ethephon and of C_2H_4. Ethephon (100 μg/ml) was neutralized with NaOH, and its effect on pigment leakage and C_2H_4 production is shown in Table I. Neutralization of Ethephon resulted in a high production of C_2H_4, but pigment leakage in this solution was no different from that in the DI control. When C_2H_4 (20 μl/l) was bubbled through the DI test solution, leakage was not significantly different from the DI control that was ventilated with C_2H_4-free air.

Effect of Added Ca^{2+}. Discs were bathed in solutions containing Ethephon or HCl at the same pH with and without 10 mM Ca^{2+}. The final pH and pigment leakage are shown in Table II. Ca^{2+} reduced leakage in 100 μg/ml Ethephon or in HCl at the same pH (3.3). However, at 1,000 μg/ml Ethephon (pH 2.3), Ca^{2+} did not reduce pigment leakage.

Time Course of Ca^{2+} and Betacyanin Leakage. Discs were placed in solutions of HCl (pH 3), and individual replicates were removed at intervals for the assay of betacyanin and Ca^{2+} leakage. After a lag of about 20 min, pigment in the test solution increased steadily to 6 h (Fig. 2). The Ca^{2+} loss from the tissue increased very rapidly in the 1st h but increased very little beyond the 3rd.

DISCUSSION

Ethephon can greatly accelerate the leakage of betacyanin from beet discs, and this leakage can be reduced (at pH 3.5) by Ca^{2+}. These observations appear to suggest that C_2H_4 released from the Ethephon changes membrane permeability and that its effects are being reversed by Ca^{2+}, which is known to retard senescence in plant systems (2). The data show that Ethephon-induced leakage is not related to the growth regulator function of Ethephon but to the low pH of its solutions. Over the pH range 2 to 4, leakage of betacyanin pigment from beet discs caused by Ethephon was the same as that caused by HCl. Moreover, when Ethephon was neutralized, leakage of betacyanin was negligible and no different from that of the DI control, even though a substantial release of C_2H_4 occurred. Treatment with 20 μl/l C_2H_4 in air was similarly ineffective in eliciting leakage of the pigment.

Ca and betacyanin both leak from beet discs held in low pH solutions, and the leakage is aggravated as the pH of the test solution drops (Fig. 1). Physiologists studying the "aging" of discs of storage tissue (including beet) often use 0.5 mM $CaSO_4$ as a medium for washing the discs (6) and have postulated that the Ca^{2+} is necessary to maintain membrane function. It can be suggested that leakiness in acid solutions results from the removal of Ca^{2+} from membranes by exchange with protons. The observation that a large proportion of the Ca^{2+} removed from the beet discs by acid is already in the solution before betacyanin leakage commences (Fig. 2) is consistent with this hypothesis, although the source of the released Ca^{2+} was not identified in this study. The reduction of leakage by addition of Ca^{2+} can be interpreted as a reduction by mass action of proton exchange with membrane Ca^{2+}.

Table I. *Effect of Ethephon, Neutralized Ethephon, and C_2H_4 on Betacyanin Leakage and C_2H_4 Concentration over Beet Discs*

Leakage of betacyanin and C_2H_4 concentration were measured for beet discs placed in various solutions. Discs placed in DI or in DI aerated with a slow stream of air containing 20 μl/l C_2H_4 served as controls.

Treatment	pH	O.D.$_{540}$	C_2H_4 Concentration
			μl/l
DI	6.6	0.003	0.02
Ethephon, 100 μg/ml	3.4	0.390	0.11
Ethephon, 100 μg/ml + NaOH	6.8	0.007	87.50
DI + C_2H_4, 20 μl/l	6.9	0.016	(20)

Table II. *Effect of 10 mM $CaCl_2$ on the Leakage of Betacyanin after 4 h from Beet Discs in Ethephon or HCl Solutions*

Solution	−Ca		+Ca	
	pH	O.D.$_{540}$	pH	O.D.$_{540}$
Ethephon, 100 μg/ml	3.35	0.53	3.27	0.14
HCl	3.56	0.36	3.25	0.10
Ethephon, 1,000 μg/ml	2.41	1.12	2.28	1.12
HCl	2.48	1.02	2.38	1.10

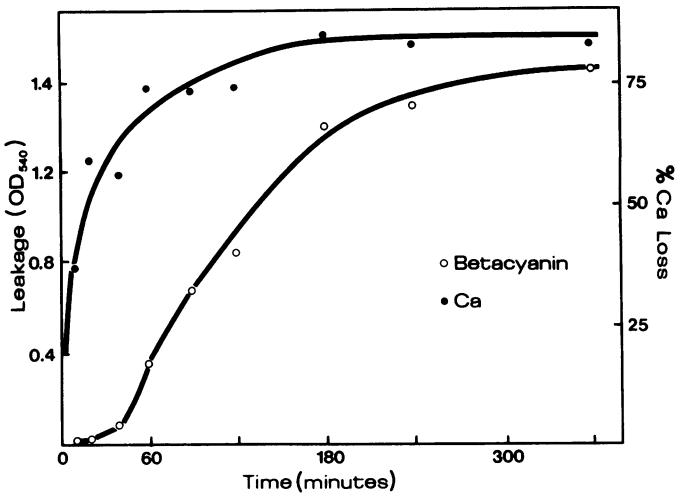

FIG. 2. Time course of betacyanin and Ca^{2+} leakage from beet discs. Replicate sets of beet discs were incubated in 1 mM HCl (pH 3). At intervals, the betacyanin and Ca^{2+} contents of the solution from individual replicates were measured.

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