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Communication

Effect of Hydrogen Cyanamide on Amino Acid Profiles in Kiwifruit Buds during Budbreak

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ABSTRACT

Buds, and resultant shoots, were collected from kiwifruit (*Actinidia deliciosa* [A. Chev.] CF Liang et AR Ferguson var *deliciosa* cv Hayward) vines from late autumn until late spring with and without hydrogen cyanamide treatment. Those samples were weighed and analyzed for total nitrogen and free amino acids. By 7 days after hydrogen cyanamide treatment, the amount of proline had risen to nearly one-quarter of the total amino acid pool in the treated buds and that proportion was maintained for at least 14 days before it declined. The maximum concentration detected in treated buds was 25.8 micromoles per gram dry weight, 6.6 times that detected in untreated buds. By 95 days after treatment, the relative amounts of proline were not significantly different.

The kiwifruit industry in New Zealand is centered on the Bay of Plenty. Vines in this area often receive suboptimal winter chilling, reducing bud break and flowering. Consequently, irregular cropping is a significant problem.

An application of calcium cyanamide or HC[1] can be used to overcome a lack of winter chilling in many plants (7). In New Zealand, HC was first applied to kiwifruit vines in 1983 (22). Since then, the use of HC has been developed into a commercial orchard management practice (9, 10). The correct timing and rate of application is important. The major benefits are (a) an increased and synchronized budbreak, and (b) increased numbers of flowers per shoot (9, 14, E.F. Walton, P.J. Fowke, unpublished). However, it is difficult to separate the events of budbreak and flower differentiation in kiwifruit, because flower differentiation commences just prior to budbreak (4).

The aim of our work is to understand how HC influences dormancy and flower differentiation in kiwifruit. This paper reports the dynamics of the amino acid pools in kiwifruit buds during and immediately following dormancy with and without HC treatment.

MATERIALS AND METHODS

Kiwifruit (*Actinidia deliciosa* [A. Chev.] CF Liang et AR Ferguson var *deliciosa* cv Hayward) buds were collected from the distal portions of canes between April 21 and November 11, 1989, from well managed vines growing on T-bars (18) in Te Puna, Bay of Plenty, New Zealand. The earliest sample was collected prior to leaf fall and the latest after the buds had broken and developed shoots. At each sampling date, five replicates of 40 buds were dissected from canes with similar diameters. Once shoots had developed, sample size was reduced to five replicates of three.

The previous season's crop was harvested on May 25 (78 d before HC treatment); leaf drop occurred between June 16 and 30 (56 and 42 d before treatment, respectively). Sap flow had commenced by July 28 (14 d before treatment), that is, the cut surfaces of the canes were moist. Treated vines were sprayed with a 5.8% product solution of HI-CANE (520 g L^{-1} HC, SKW Trostberg AG, Germany) with an air-blast sprayer, applied at a rate of 794 L ha^{-1} on August 11, after sample collection that day. Subsequent samples were collected at a similar time of day.

Total N and free amino acids were measured after the samples had been lyophilized and ground to a fine powder.

Nitrogen was analyzed by Kjeldahl analysis (20).

Amino acids were extracted from a 0.5 g dry weight sample for 24 h on a shaker at ambient temperatures with 50 mL of a single phase solution of methanol:chloroform:water (12:5:3, v/v), after Bieleski and Turner (3). Norleucine was added (0.5 mL, 4 mM solution in 0.01 N HCl) prior to extraction as an internal standard.

After extraction, the colorless aqueous methanolic phase (containing the amino acids) was separated from the chloroform phase (containing pigments and lipids) (see 16). The solid residue was gently simmered for 10 min in 40 mL water to extract any residual amino acids (15). On cooling and centrifugation, the aqueous extract was combined with the methanolic extract and made up to 100 mL.

A 20 mL aliquot was loaded onto a cation-exchange column (Dowex-50W 8%, 200–400 mesh) with a bed volume of 3 mL. That column was then washed with 45 mL 0.01 N HCl, followed by 5 mL water, and then eluted with 30 mL 2 N NH_4OH to release the amino acids. Flow rates were maintained at approximately 1 mL min^{-1} with a small vacuum pump. (Columns were regenerated by sequentially washing with 5 mL water, 10 mL 1 N HCl, 5 mL water, 40 mL 0.2 N NaOH, 5 mL water, 10 mL 1 N HCl, and 5 mL water, after Lazarus [13]).

The ammoniacal eluates were lyophilized and stored at −10°C until analysis, at which time they were resolubilized in 0.2 M lithium citrate loading buffer (pH 2.20, LKB Biochrom). Amino acids were separated on an Alpha Plus amino acid

[1] Abbreviation: HC, hydrogen cyanamide.

analyzer (LKB Biochrom) using an unmodified physiological analysis program. Ninhydrin-positive compounds were generally identified by retention time, but on occasion identification was by comparing the peak area ratios of absorbances measured simultaneously at 440 and 570 nm (23).

RESULTS AND DISCUSSION

Kiwifruit vines treated with HC started growing earlier than the untreated vines (Fig. 1A). Bud break (the time when 10% of the buds had developed shoots 10 mm long) occurred 37 d after treatment with HC, 7 d prior to the untreated plants. By 95 d after spraying treated vines, however, there was no significant difference in shoot dry weight between treated and untreated plants. Nitrogen concentrations were stable while dormant (mean 4.6 mg g^{-1} dry weight, SE = 0.57), rose prior to the increase in dry weight, peaked at 49 d after treatment in both treated and untreated plants, and then declined (Fig. 1B). Unlike dry weight, nitrogen concentrations were not significantly different between treated and untreated plants from 49 d after treatment.

The concentration of total amino acids increased in the treated plants before the untreated plants, but was greatest in both groups 49 d after treatment (Table I), shortly after budbreak. The same trends were recorded for individual amino acids, except that Pro was at its greatest concentration in the treated plants 28 d after treatment, although not significantly higher than the concentration at 49 d after treatment. In the treated plants, there were small, transient peaks (appearing 1 d after HC treatment and lasting 1 d) of Gln, Gly, Ser, and Val (data not shown). It is assumed that the plant was scavenging the nitrogen from the HC, as has been shown by Amberger (1). The ratio of Glu to Gln in the buds changed from greater than 1 while dormant, to lower than 1 when actively growing. This may prove a useful indicator of the breaking of dormancy prior to physical changes being observed. As development proceeded, additional amino acids were detected in small quantities, starting 7 d after treatment in the treated plants and 14 d after treatment in the untreated plants. Those were Ile, Leu, Tyr, Phe, Orn, Lys, 1-methylhistidine, and His (data not presented).

Pro was first detected 7 d after treatment with HC. By that time, the amount of Pro was nearly one-quarter of the total amino acid pool and that proportion was maintained for at least 14 d before it declined (Fig. 1C). In relative terms, the maximum amount detected in untreated buds was 3.8% of the amino acid pool, at 28 d from treatment. The maximum concentration detected in treated buds was 25.8 μmol g^{-1} dry weight, 6.6 times that detected in untreated buds (Table I). By 95 d after treatment, the concentrations and relative amounts of Pro were not significantly different between treated and untreated plants (Table I and Fig. 1C, respectively). Other amino acids also showed significant increases in their peak concentrations when treated with HC, namely Ser (34%), Gly (208%), Ala (19%), and γ-aminobutyrate (53%).

In kiwifruit, flower primordia are not present in the buds during dormancy, but begin to differentiate at bud swell, approximately 10 to 15 d prior to budbreak (4). The rise in the relative concentrations of Pro in the buds of treated plants

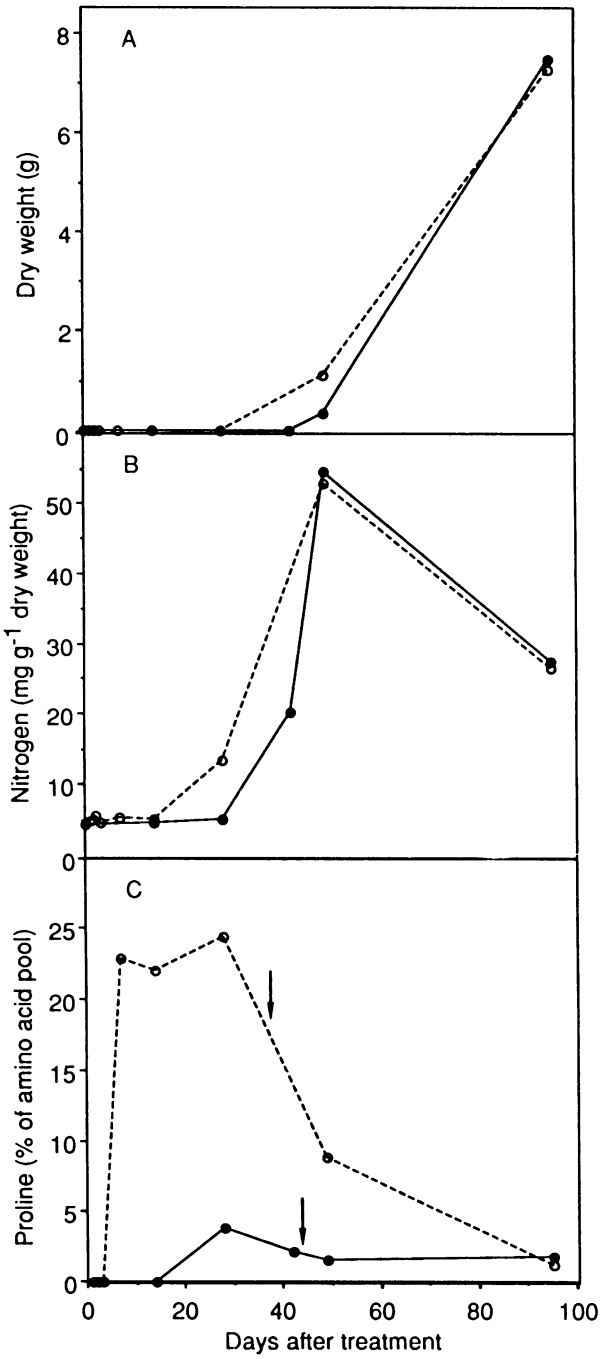

Figure 1. Dry weight (A), nitrogen (B), and Pro (C) of kiwifruit buds from plants treated (– – –) and untreated (—) with HC. The arrows on C indicate the timing of budbreak. Maximum SE: dry weight, 0.63; nitrogen, 1.6; Pro, treated, 0.13, untreated 1.7.

Table I. *Principal Amino Acids Extracted from Buds of Kiwifruit Plants Treated and Untreated with HC*

Amino Acid	Amount Extracted (Days from Treatment)													
	Pretreatment			Untreated				Treated						
	−112	−56	0	14	28	49	95	1	3	7	14	28	49	95
							μmol g⁻¹ dry wt							
Total[a] (μmol g⁻¹ dry wt)	10.45	4.53	4.02	6.22	26.3	254	59.4	5.17	2.73	9.77	17.8	101	280	47.9
SE	0.60	0.25	0.32	0.04	2.1	14	2.5	0.15	0.10	0.26	0.77	6.5	6.1	1.5
Asp	2.28	1.07	0.78	0.89	0.70	18.4	5.07	1.04	0.46	1.70	1.48	4.86	14.9	4.66
Thr	0.20	0.12	T[b]	0.13	0.53	3.84	1.37	T	T	0.12	0.25	0.80	5.32	1.24
Ser	0.46	0.28	0.24	0.28	1.21	11.2	3.54	0.26	0.16	0.41	0.72	3.24	15.0	3.40
Asn	1.48	0.19	T	0.27	0.90	43.1	7.34	T	T	T	0.71	3.38	38.9	5.36
Glu	2.35	1.20	0.67	1.66	7.08	24.1	8.15	0.90	0.54	1.90	3.51	15.8	21.6	7.58
Gln	0.57	0.11	T	0.28	3.76	100	12.6	0.36	0.48	1.15	1.98	26.8	92.1	8.43
Pro	0	0	0	0	1.00	3.89	1.04	0	0	2.26	3.94	25.8	25.1	0.57
Gly	0.20	0.18	0.19	0.16	0.32	1.19	0.49	0.26	0.16	0.24	0.35	1.11	2.47	0.40
Ala	0.78	0.51	0.77	0.83	5.70	9.57	5.28	0.87	0.44	0.79	1.84	10.6	11.4	4.58
Val	0.48	0.35	0.29	0.34	1.18	11.4	4.54	0.34	0.24	0.34	0.66	3.77	11.6	4.19
γ-Aminobutyrate	0.91	0.31	0.71	0.82	1.94	9.35	6.55	0.73	0.25	0.50	1.27	6.00	14.3	4.76
Arg	0.74	0.21	0.37	0.31	0.82	10.4	0.66	0.41	T	0.18	0.29	1.69	13.7	0.54
Mean CV[c]	27.1	18.8	21.0	12.6	18.6	13.2	12.9	10.1	14.6	10.0	17.3	20.4	9.6	15.7

[a] Differences between sums of the individual amino acids and the totals reflect the additional amino acids that accumulate in small quantities during budbreak. [b] T = trace (<0.1 μmol g⁻¹ dry wt). [c] For individual amino acids, SE = (mean CV · $\bar{x}$)/100$\sqrt{5}$).

occurred at least 30 d prior to budbreak and, therefore, prior to the start of flower differentiation (Fig. 1C). In the buds of untreated plants, however, the rise in Pro had occurred by only 16 days before budbreak. The relative concentrations of proline were declining in both treated and untreated plants prior to budbreak and, therefore, prior to anther and pollen grain formation, which occur between 35 and 40 d after budbreak (4). It should be noted, however, that in functionally pistillate kiwifruit clones, the nucleus and cytoplasm of the pollen degenerate during development so that it is nonviable by dehiscence (17). Because Pro does not provide a significant contribution to the amino-nitrogen status of the plant (5), it is possible that Pro accumulation is associated with the onset of flowering in kiwifruit, as with other species (2). The earlier increases and greater concentrations of Pro in the buds of HC-treated plants could favor the observed increase in bud fruitfulness (14, E.F. Walton, P.J. Fowke, unpublished).

Pro accumulation in plant tissues has also been linked to many environmental stresses, including mineral deficiencies, salinity, drought, flooding, extreme temperatures, and pathogen attack (for review, see ref. 21). The Pro data presented here could be interpreted as HC increasing budbreak through sublethal stresses (7), particularly because HC at high rates can damage kiwifruit canes (10).

The activation of the pentose-phosphate pathway is thought to be necessary for the breaking of dormancy in buds and seeds (19). Glucose-6-phosphate dehydrogenase, which catalyzes the rate limiting step in the pentose-phosphate pathway, is not only dependent on the availability of $NADP^+$, but is inhibited by NADPH (6). To keep the pentose-phosphate pathway functioning, NADPH would need to be consumed, and this could be done by reducing Δ^1-pyrroline-5-carboxylate to Pro (8). This type of interaction between Pro and the pentose-phosphate pathway has been documented in legume root nodules (11, 12). Elevated levels of Pro in HC-treated plants could be associated with a greater stimulation of the pentose-phosphate pathway, resulting in a greater percentage of budbreak (9, 14) and increased bud fruitfulness (14, E.F. Walton, P.J. Fowke, unpublished).

Research is continuing to identify the physiological significance of Pro accumulation in kiwifruit buds treated with HC.

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