

## Increase of the acid contents in grape berries by treatment with cAMP

by

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### Zunahme des Säuregehaltes reifender Traubenbeeren durch Behandlung mit cyclischem Adenosin-3',5'-monophosphat

**Zusammenfassung.** — Bei der Rebsorte Delaware wurde durch Behandlung der Infloreszenzen mit cAMP vor der Blüte der Gehalt des Beerensaftes an organischen Säuren erhöht.

Durch cAMP-Behandlung nach der Blüte wurde der Gesamtgehalt der Aminosäuren gesteigert, wobei Alanin, Arginin und  $\gamma$ -Aminobuttersäure zunahmen, während die Tyrosinkonzentration verringert war.

### Introduction

In a previous paper, we reported that adenosine-3',5'-monophosphate (cAMP) applied together with gibberellic acid ( $GA_3$ ) to the developing inflorescences increased the seedless fleshy berries in Campbell Early grapes (*Vitis labruscana* BAILEY). This was considered to result from the delaying effect of cAMP on the decrease of applied  $GA_3$  activity, although such a process was difficult to explain (5).

Recently, WIEDMAIER and ULRICH found that cAMP application to green leaves of *Petunia hybrida*, *Impatiens sultani* and *Coleus blumei* increased the foliar content of fructose and stimulated the photosynthesis (6).

In this paper, we report the effects of cAMP only, applied to the developing inflorescences of grapes, on the contents of organic acids, sugars and amino acids in the juice of mature berries.

### Materials and methods

Experiments were carried out over three years (1976 to 1978) in the vineyard of the Yamagata Horticultural Experiment Station in Sagae-Shi using two vines of the cultivar Delaware (*Vitis labruscana* BAILEY). The plants were 12 years old in the first year of the experiment. Inflorescences were thinned out before the application of cAMP, leaving two inflorescences on a current shoot.

cAMP solution was prepared by dissolving cAMP sodium (Kikkoman Shoyu Co., Ltd., Noda-Shi) in deionized water to the desired concentrations. Aerol OP (Toho Co., Ltd., Tokyo) was added in 100  $\mu$ g/ml as a wetting agent. The solution was applied to the inflorescences by spraying or dipping. The prebloom application was carried

out about 15 d before the expected full bloom of the untreated inflorescences, and the postbloom application about 10 d after full bloom.

All clusters reached maturity at almost the same time regardless of the treatments, and 10 fruit clusters, which were regarded to show the best fruit set in the respective treatments, were harvested simultaneously unless stated otherwise.

Each of 10 ripened fruit clusters in the different treatments was squeezed separately with a juicer of Hitachi-VJ-100 type (Hitachi-Seisakujo Co., Ltd., Hitachi-Shi). The juice obtained was filtered with Toyo-filter paper No. 2 (Toyo Co., Tokyo). Titratable acids were measured by adding 0.1 n sodium hydroxide to 2 ml of the filtered juice (diluted with deionized water to 20 ml), until a pH meter showed pH 8.2; it is expressed as ml alkaline consumed. The contents of tartaric and malic acid, as the principal organic acids in grape juice, were determined with a carboxylic acid analyzer (Seishin-Seiyaku Co., Tokyo) (4) applying the filtered juice directly. Amino acids were estimated by an amino acid analyzer of Hitachi-KLA-5 type (Hitachi-Seisakujo Co., Ltd.). Reducing sugars were measured by BERTRAND'S method modified by HENMI and TOMOEDA (2).

Values of titratable acids and reducing sugars of cleared juice obtained separately on each of 10 fruit clusters were expressed as their means. In case of organic acids and amino acids, aliquots of the cleared juice from each of 10 fruit clusters were combined in the respective treatments before measuring.

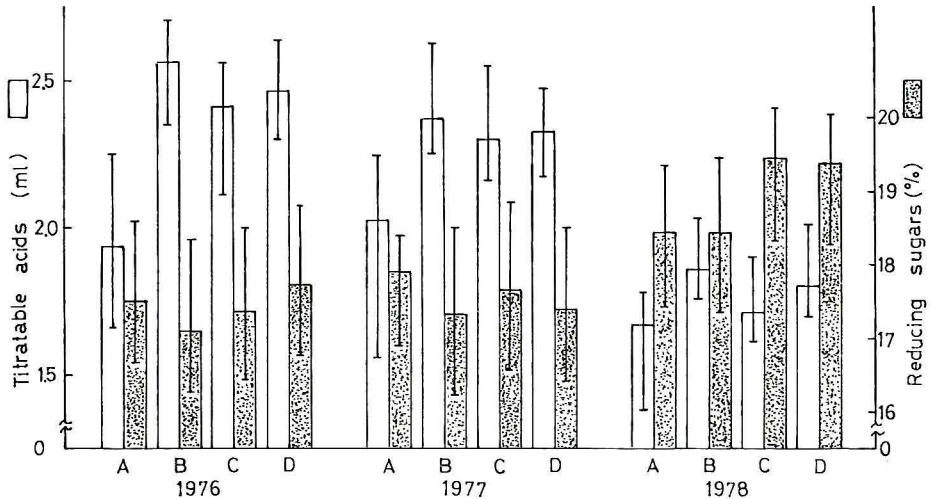


Fig. 1: Effects of cAMP sprayings (500  $\mu\text{g}/\text{ml}$ ) and seasons on the titratable acids and reducing sugars in fruit clusters of the cultivar Delaware. The vertical bars indicate the highest or the lowest values among 20 grape clusters in each treatment. A = control (no application), B = prebloom, C = postbloom, D = pre- and postbloom application.

Einfluß der cAMP-Behandlung (Sprühen von 500  $\mu\text{g}/\text{ml}$ ) und des Jahrganges auf die gesamte titrierbare Säure und die reduzierenden Zucker in Trauben der Sorte Delaware. Die senkrechten Strecken bezeichnen die höchsten bzw. niedrigsten Werte für jeweils 20 Trauben. A = Kontrolle (keine Behandlung), B = Behandlung vor, C = Behandlung nach, D = Behandlung vor und nach der Anthese.

**Results**

Fig. 1 shows the effects of cAMP application to inflorescences by spraying on titratable acids and reducing sugars of fruits that were obtained from 1976 to 1978. Fig. 2 indicates the distribution of titratable acids values in 50 fruit sets. In the above data, pre- and/or postbloom application of cAMP caused a significant increase

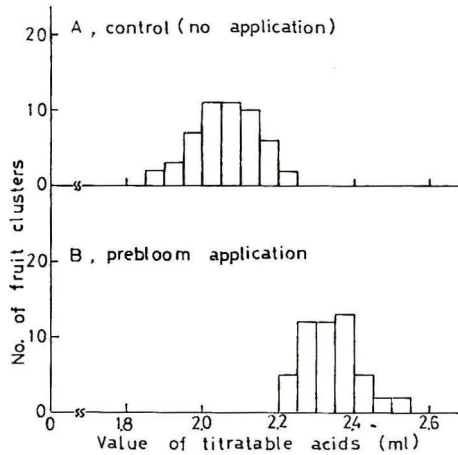


Fig. 2: Distribution of titratable acid values in 50 fruit clusters. Application of 500 µg cAMP/ml by spraying.

Häufigkeitsverteilung der Werte der gesamten titrierbaren Säure bei 50 Trauben. Sprühen von 500 µg cAMP/ml.

**Table 1**

Effects of cAMP on the organic acid contents in juice of ripe berries of Delaware (1978)  
Einfluß von cAMP auf den Gehalt an organischen Säuren im Saft reifer Beeren der Sorte Delaware (1978)

cAMP application (µg/ml)		pH	Reducing sugar (%)	Acidity (ml)	Tartaric acid (g/l)	Malic acid (g/l)	Tart. + mal. acid (g/l)
Prebloom	Postbloom						
—	—	3.35	18.45	1.67	2.30	3.03	5.33
500 (S)	—	3.35	18.45	1.83	3.58	3.77	7.34
—	500 (S)	3.39	19.22	1.70	2.50	3.30	5.90
500 (S)	500 (S)	3.40	19.38	1.80	3.45	3.67	7.12
500 (D)	—	3.30	19.53	1.80	3.37	3.67	7.04
—	500 (D)	3.30	19.22	1.78	3.29	3.58	6.87
500 (D)	500 (D)	3.39	19.22	1.78	3.18	3.48	6.66

— = No application.  
S = Spraying treatment.  
D = Dipping treatment.

in the titratable acids as compared with the control, though the content of reducing sugars was variable with years. The higher values of titratable acids for 1976 and 1977 seemed to be due to rains and inclement weather prevailing during berry development and harvest.

Table 1 and 2 indicate the results of 1978 and 1977 experiments, respectively. From Table 1 it can be recognized that there were appreciable increases of tartaric and malic acid contents corresponding to the rise in titratable acid values in the fruits as compared with the control. In contrast, Table 2 shows that the total content of amino acids was increased only by postbloom application.

The increase of the organic acid content by prebloom application could be recognized in every season over three years independently of the methods of application (spraying or dipping) and of cAMP concentrations in the range of 100 to 500  $\mu\text{g/ml}$ . The effects of postbloom application on the organic acid content, however, were variable with years and with application methods.

Among amino acids, contents of alanine, arginine and  $\gamma$ -amino-butyric acid were increased, but that of tyrosine was decreased in the fruits (see Table 2).

cAMP application gave hardly some effects on the growth of berries and clusters of grapes.

Table 2

Effects of cAMP on the amino acid composition in juice of ripe berries of Delaware (1977)  
Einfluß von cAMP auf die Aminosäurezusammensetzung im Saft reifer Beeren der Sorte Delaware (1977)

Amino acids ( $\mu\text{g/ml}$ )	cAMP application (500 $\mu\text{g/ml}$ )			
	Control	Prebloom	Postbloom	Prebloom + postbloom
Aspartic acid	41	33	40	34
Threonine	60	67	72	64
Serine	249	260	342	267
Glutamic acid	93	90	116	93
Glycine	13	9	17	10
Alanine	518	555	671	545
Valine	73	68	72	77
Methionine	ND	ND	11	ND
iso-Leucine	15	15	15	16
Leucine	17	34	35	32
Tyrosine	148	28	31	32
Phenylalanine	91	81	87	83
$\gamma$ -Aminobutyric	239	269	315	271
Lysine	12	ND	ND	ND
NH <sub>3</sub>	48	58	64	53
Histidine	ND	ND	15	ND
Arginine	286	386	478	356
Total amount	1848	1894	2303	1871

ND =No detection.

### Discussion

The increase of acid content of vine berries together with a certain level of reducing sugars is important for wine and juice making.

From the results described above, and the presence of cAMP in inflorescences of the grapevine cultivar Delaware, reported in our previous paper (5), it is suggested that cAMP might possibly play a part in the berry development of grapes.

It was, however, not yet determined whether the effects of cAMP, described above, were caused by cAMP itself or by its decomposition products (adenosine 3'-, or 5'-monophosphate, inosine 3'-, or 5'-monophosphate, adenosine, inosine, adenine, hypoxanthine, xanthine, etc.) by enzymes (phosphodiesterase, deaminase or phosphatase, etc.), after cAMP had been incorporated into the clusters.

Some reports (1, 2), however, had demonstrated that the application of such compounds as adenine, xanthine, caffeine and uracil, at the prebloom-phase of vines of some varieties, when the shoots were 30—40 cm long, caused the increase of RNA synthesis of leaves, fruitfulness and sugar content of the berries of the treated vines, but produced no change in the acid content of the berries and the time of fruit maturity. Therefore, it is conceivable that the action of cAMP itself might presumably bring about the increase of acid content.

As organic acid content of berries is closely related with maturity, the high content of organic acid might be possibly due to the delay in maturation, although in the content of reducing sugars, as an index of maturity, differences between the treatments in the 1978 experiments could not be found (see Table 1). cAMP also might promote the translocation of organic acids from leaves into berries due to the alternating activities of enzymes, involved in metabolism of carbohydrates, organic acids and amino acids, which were induced by cAMP itself.

However, in order to elucidate clearly the increase of organic acid content in grape berries by cAMP treatment, further experiments must be carried out with regard to maturity by examining respiration, photosynthesis and enzyme activities, affected by cAMP or its decomposition products.

### Summary

cAMP prebloom application to flower clusters of the grapevine variety Delaware (*Vitis labruscana* BAILEY) raised the content of organic acids in the reopening berries. The total content of amino acids in the juice was raised by postbloom application, alanine, arginine and  $\gamma$ -aminobutyric acid being increased, whereas tyrosine was decreased.

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