Phytochemistry, 1971, Vol. 10, pp. 1779 to 1781. Pergamon Press. Printed in England.

SHORT COMMUNICATION

ORGANIC ACID SYNTHESIS BY GRAPE BERRIES CULTURED *IN VITRO*

K. G. M. SKENE and C. R. HALE

C.S.I.R.O., Division of Horticultural Research, G.P.O., Box 350, Adelaide, South Australia 5001, Australia

(*Received* 8 *September* 1970)

Abstract—Inflorescences of grape (*Vitis vinifera* L. cv. Zante Currant) were excised a few days before flowering and cultured on synthetic media. Berries developed slowly; they contained normal concentrations of malic acid, but no tartaric acid could be detected.

INTRODUCTION

MALIC and tartaric acids, the two main organic acids in the fruit of the grape (*Vitis vinifera* L.), accumulate to high levels during certain stages of berry development.[1] Growth of the grape berry is biphasic, two periods of rapid growth being separated by a lag phase or period of slow growth. The amounts of both tartaric and malic acids in the berry rise during the first rapid growth phase; malate begins to fall at the start of the second rapid growth phase, at the onset of sugar accumulation, whereas the amount of tartrate remains relatively constant.[2]

The results of ^{14}C incorporation studies[3–6] indicate that immature grape berries are capable of synthesizing malic and tartaric acids, either from sugars supplied to excised berries,[4] or from CO_2-fixation products of the berry[6] or leaf.[3] However, the quantitative importance of the berry as a site of organic acid synthesis is not fully established, and it is possible that these acids are also translocated to berries from other parts of the plant.

This communication reports on the results of culturing grape berries *in vitro* from inflorescences excised before anthesis. The berries contained normal levels of malic acid, but no tartaric acid could be detected.

RESULTS AND DISCUSSION

Within a few days of excision, inflorescences in all culture tubes flowered. Many flowers set fruit, but subsequent berry growth proceeded very slowly. After 3 months culture, most of the berries were still quite hard and green. A few berries had started to soften, indicating the beginning of the second rapid growth phase, although in no case was there evidence of red pigmentation, which normally occurs in this cultivar during ripening.

[1] W. M. KLIEWER, *Am. J. Enol. Vit.* **16**, 92 (1965).
[2] C. R. HALE, *Australian J. Agri. Res.* **19**, 939 (1968).
[3] C. R. HALE, *Nature* **195**, 917 (1962).
[4] P. J. HARDY, *Plant Physiol.* **43**, 224 (1968).
[5] K. SAITO and Z. KASAI, *Plant Cell Physiol.* **9**, 529 (1968).
[6] K. SAITO and Z. KASAI, *Phytochem.* **8**, 2177 (1969).

TABLE 1. SUGAR AND ORGANIC ACID CONTENT OF GRAPE BERRIES CULTURED *in vitro*

Sample	Berry dia. (mm)	Berry fr. wt. (mg)	Berry condition	Sugar (as % fr. wt) Non-reducing	Sugar (as % fr. wt) Reducing	Organic acid (as % fr. wt) Malate	Organic acid (as % fr. wt) Tartrate	Reducing sugar/malate ratio
Cultured berries								
1	3	20	hard, green	—	—	1·36	0	
2	3·5	30		—	—	0·98	0	
3	3	20		2·2	5·0	1·00	0	5·0
4	3	26		1·1	3·4	0·73	0	4·7
5	4	50		1·4	4·1	0·85	0	4·8
6	4	54	soft, green	1·7	7·6	0·34	0	22·4
Field-grown berries								
7	7·7	270	hard, green	—	—	1·73	1·07	
8	7·8	290	soft, red	—	—	0·30	0·80	

Culturing berries in a 16-hr photoperiod at 25° made no difference to rates of growth, pigment formation, or to the results reported below.

All berries contained non-reducing sugars, reducing sugars and malic acid (Table 1). However, no tartaric acid was detected in any sample of cultured berries. The increase in reducing sugars, the drop in malic acid and the concomitant increase in the reducing sugar/malate ratio of sample 6 confirm the observation that ripening had commenced in this berry. Paper chromatography indicated that glucose and fructose were present in approximately equal proportions in extracts of all the cultured berries. In contrast to cultured berries, both malic and tartaric acids were detected in extracts of berries developed *in vivo* (samples 7 and 8), the malic acid levels being comparable to those of cultured berries.

The fact that cultured berries contained no tartaric acid, but were capable of accumulating malic acid to concentrations similar to those of field-grown berries, could indicate that in the intact plant most of the tartaric acid, as distinct from the malate, is transported to the berries from other organs. However, it is apparent from the slow growth rates that berry development was not entirely normal. Restricted berry growth and the absence of tartaric acid may be a reflection of the medium's deficiencies; tartaric acid accumulation is usually associated with rapidly growing tissues,[7,8] and it is only recently that tartrate biosynthesis in plants has begun to be understood.[6]

EXPERIMENTAL

Berry Culture Conditions

Inflorescences of *Vitis vinifera* L. cv. Zante Currant were selected from glasshouse-grown plants a few days before flowering. After shaking for 10 min in clarified 5% $Ca(OCl)_2$, inflorescences were separated into clusters of about 20 flowers and transferred to culture tubes containing White's medium[9] in which Fe^{+++} EDTA replaced $Fe_2(SO_4)_3$. The medium was set with 0·5% agar and supplemented with 1 mg/l. α-naphthaleneacetic acid. Tubes were placed in a growth cabinet maintained at 23° with an 8 hr photoperiod. After 2 months, berries were transferred to fresh culture tubes in which the sucrose concentration of the medium was increased from 2 to 5%. Samples for analysis were removed after a further month.

[7] W. M. KLIEWER, *Plant Physiol.* **39**, 869 (1964).
[8] W. M. KLIEWER and A. R. NASSAR, *Am. J. Enol. Vit.* **17**, 48 (1966).
[9] P. R. WHITE, *The Cultivation of Animal and Plant Cells*, Ronald Press, New York (1954).

Analysis of Sugars and Organic Acids

Individual washed berries were extracted in boiling water; total sugars in the extracts were assayed using anthrone reagent,[10] and reducing sugars by the method of Somogyi.[11] Organic acids were assayed by GLC of their trimethylsilyl derivatives,[12] using an internal standard of β-methyl β-*n*-propyl glutaric acid.

Acknowledgements—Mrs. J. M. Smith and Mr. B. W. F. Dolkens provided competent technical assistance.

[10] F. A. Loewus, *Anal. Chem.* **24**, 219 (1952).
[11] M. Somogyi, *J. Biol. Chem.* **195**, 19 (1952).
[12] Z. Horii, M. Matika and Y. Tamura, *Chem. & Ind.* 1494 (1965).