Vitis **10**, 210—214 (1971)

Division of Horticulture, Agricultural College and Research Institute, Coimbatore, India

Nucleic acid composition in the developing buds and petioles of grapes

by

V. N. Madhava Rao and C. Srinivasan

Introduction

Buds in grapes are formed as discrete tissues in the axils of leaves subtended by the current season's shoots. During their development, these buds may differentiate into cluster bearing or vegetative primordia. Past work in the Division of Horticulture, Agricultural College and Research Institute, Coimbatore has shown that the initiation of inflorescence primordia occurs about 45 days after bud burst in the cultivar Anab-e-Shahi (Rajaram *et al.* 1965). Subsequent work showed that application of P_2O_5 at the time of flower initiation (Nanaya 1966) and K_2O (Manivel 1967, Srinivasan 1968) favoured formation of cluster primordia. In order to study the role of other factors in fruit bud formation, the relationship between nucleic acid and amino acid composition of buds and petioles at different periods of development of the shoot was taken up for investigation. The first part dealing with the nucleic acid composition is presented here.

Histochemical changes connected with nucleic acid metabolism have been demonstrated in the shoot apex of *Chenopodium album* before the morphological changes in flower initiation became evident (Gifford and Tepper 1962). Kessler *et al.* (1959) showed in the grape vine that application of 50 ppm uracil increased the flower which is parallelled by an increase in protein N and RNA/DNA ratio. Recently, Ćeban (1968) observed an increased accumulation of nucleic acids during the period of differentiation and their synthesis retarded during dormancy. Jako (1970) reported an increase in DNA and RNA of vine leaves treated with uracil but a decrease in DNA when adenine was applied.

Materials and Methods

Buds and petioles (which reflect the nutrient status of grape vines) from bearing vines of Anab-e-Shahi were collected 15 and 30 days after bud burst, subsequently at 5 day intervals from 30 to 60 days (period of initiation and differentiation) and at monthly intervals 90 and 120 days after bud burst. Buds and petioles on the second, fifth and tenth nodes were collected and analysed separately. The productive and vegetative shoots were sampled at bud burst when the inflorescence was just discernible. The dormant buds collected 150 days after bud burst were classified into vegetative[1]) and productive[2]) buds by dissecting under a steriomicroscope, and the whole buds were utilized for analysis. For the extraction and fractionation of the nucleic acids into component Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA), the method of Nieman and Poulson (1963) was followed with suitable modifications in extraction. The sediment, precipitated from the frozen buds and petioles weighing 0.5 g with cold ethanol and centrifugation was resuspended in 0.3 N NaOH and incubated at 37° C for 16 hours. Care was taken to

[1]) (Buds lacking inflorescence primordia).
[2]) (Buds carrying inflorescence primordia).

remove the traces of ethanol in the sediment by suspending with diethyl ether 4 or 5 times. The solution was then acidified to pH 1.0 with 15 per cent (v/v) perchloric acid. After incubating for 60 minutes at 4° C, the precipitate was centrifuged down and washed once again with a 1 N perchloric acid. The combined supernatant fluids constituted the RNA fractions. The DNA was hydrolyzed from the residue with 0.5 N perchloric acid at 70° C for 15 minutes. Both RNA and DNA fractions were estimated spectrophotometrically by measuring the extinction of the extract at 260 nm with a Beckman DU spectrophotometer and expressed as nucleic acid phosphorus.

Results

The results (Table 1) indicated that the DNA content in buds followed identical pattern of fluctuations during their development irrespective of the bud positions. The DNA multiplications in the buds increased perceptibly from the 30th day after bud burst, the quantity being high in the fifth bud compared to the second and tenth buds. A single peak in the curve denoting a sharp increase in DNA multiplication from the 30th day and a reduction after the 40th day coincided with the period of normal flower bud initiation in the buds of Anab-e-Shahi. Accumulation rate of DNA increased little on 50 and 60 days in 5th bud but after 90 days i. e., after the comparative cessation of differentiation, there was a slight fall in all the buds.

RNA synthesis, on the other hand, decreased from bud burst to the 40th day after bud burst and increased thereafter for another five days, becoming more or less constant from 45th to 60th day and declined thereafter. The nucleic acid content

Table 1

DNA-P and RNA-P composition in the grape buds during their growth and development

S.No.	Stages of sampling	DNA-P content µg/g of fresh weight			RNA-P content µg/g of fresh weight		
		2nd bud	5th bud	10th bud	2nd bud	5th bud	10th bud
1.	At bud burst						
	(a) Productive shoot		70.3			721.3	
	(b) Vegetative shoot		62.1			371.7	
2.	15 days after bud burst	34.46	32.75	—	341.73	554.91	—
3.	30 — do —	28.45	26.70	23.25	—	317.60	—
4.	35 — do —	30.15	47.40	27.60	252.50	263.60	291.30
5.	40 — do —	50.00	54.30	49.10	185.93	174.83	266.40
6.	45 — do —	31.00	38.80	34.50	227.55	271.95	268.08
7.	50 — do —	31.90	39.90	34.50	235.85	288.60	263.83
8.	55 — do —	29.30	34.50	32.70	246.98	302.47	291.38
9.	60 — do —	24.10	41.10	27.60	227.56	277.50	321.90
10.	90 — do —	23.30	37.90	26.70	105.45	129.37	149.85
11.	120 — do —	22.40	35.30	25.90	97.13	127.65	138.75
12.	150 — do —						
	(a) Productive bud		24.33			467.80	
	(b) Vegetative bud		20.36			378.70	

Table 2

DNA-P and RNA-P composition in the petioles during their growth and development in grapes

S.No.	Stages of sampling	DNA-P content µg/g of fresh weight			RNA-P content µg/g of fresh weight		
		2nd node	5th node	10th node	2nd node	5th node	10th node
1.	At bud burst	—	—	—	—	—	—
2.	15 days after bud burst	13.80	21.55	—	197.77	554.91	—
3.	30 — do —	13.80	21.05	15.50	153.70	310.70	324.50
4.	35 — do —	11.20	16.35	8.60	97.10	166.40	117.90
5.	40 — do —	12.20	14.60	17.20	44.40	83.25	80.48
6.	45 — do —	5.17	12.20	4.30	66.60	47.18	63.83
7.	50 — do —	7.76	10.31	8.62	69.38	77.70	61.05
8.	55 — do —	6.00	11.20	7.76	72.15	86.03	66.60
9.	60 — do —	16.90	12.90	7.70	40.85	38.85	63.83
10.	90 — do —	15.50	10.30	6.90	36.20	30.50	52.70
11.	120 — do —	15.70	8.60	6.90	27.75	30.52	47.17

was generally higher during the earlier stages of growth (until 40 days) than during the subsequent periods of growth. Further, the nucleic acid content of productive buds was higher than that of vegetative buds.

In petioles, no definite pattern of fluctuations in DNA content was observed (Table 2) but the 5th nodal petioles generally contained more of DNA than the other petioles. The synthesis of RNA showed a distinct pattern of decrease from bud burst to 120 days thereafter.

Discussion

The results indicate that the DNA and RNA synthesis differs in the buds and petioles during the growth and development. DNA multiplication increased rapidly from bud burst to 40 days with a peak on the 40th day and fell thereafter. This period is the crucial time of flower bud initiation and differentiation in this variety of grapes. DNA multiplication considerably accelerated during the initiation period showing its role in this vital phase of vine's development. Bonner and Zeevart (1962) showed in *Pharbitis nil* that DNA multiplication is an essential prerequisite for floral induction, while Chinoy (1967) demonstrated accelerated multiplication of DNA during floral induction. The upsurge of DNA multiplication could be noted during floral induction in all the buds (2nd, 5th and 10th nodes). There were no significant differences among the different buds located at the 2nd, 5th and 10th nodes; but a slight increase in the DNA content was, however, noted in the 5th bud, which is generally more fruitful than the others, yielding comparatively larger bunches in this variety of grapes.

In general, the dormant buds accumulated less DNA than the developing buds. Such a retardation of nucleic acid synthesis in dormant buds was reportd by Čeban (1968). Moreover, the reproductive and vegetative shoots analysed just after bud burst showed that the reproductive shoots accumulated more DNA than the vegeta-

tive shoot. This may be due to the intense activity of the cells, during the formation of secondary and tertiary rachis of the inflorescence primordia.

RNA synthesis, on the other hand, did not correspond with DNA multiplication. RNA synthesis was high during the preinitiation period, fell considerably on the 40th day (period of initiation) after bud burst and rose thereafter up to the 45th day (period of differentiation). ENGLERT-DUJARDIN and SIRONVAL as quoted by CHINOY (1967) showed that the RNA content of chloroplast decreased at the time of floral induction. Reduction in the plastidial RNA was attributed to flower formation. In this context it may be pertinent to consider the structure of grape buds. Grape buds are composite in nature, consisting of a condensed primordial shoot with primordial leaves, initiating the inflorescence primordia at a ontogenetically fixed position opposite to the leaf primordia on the 5th or 6th nodes in the case of Anab-e-Shahi (RAJARAM *et al.* 1965). The bud tissues used for analysis contained mainly vegetative organs such as leaf primordia and bract primordia besides the floral tissue, so that the plastidial RNA present in these vegetative organs probably lowered the total RNA content. With the accumulated activity of the floral tissues from the 45th day, the RNA content appears to have been enhanced.

This postulation is strengthened by the observation in RNA content in the just burst out shoots analysed at bud burst, and the dormant buds (150 days after bud burst). Unlike buds which are in meristematic activity due to floral induction, initiation and differentiation, the petiole, a vegetative plant part stores and translocates food materials and other substances. It is suggested that due to this mobile function, there is a high fluctuation in DNA and RNA contents. The latter decreased progressively due to the continuous accumulation of dry matter content and the active cells were changed into dormant tissues.

Summary

The nucleic acids content in grape buds showed a definite pattern of fluctuations. DNA multiplication was found to be high during the initiation and early differentiation period while the RNA was high during pre-initiation period, fell considerably during initiation and rose again during differentiation. DNA multiplication in petioles did not show any definite pattern, but RNA decreased progressively from bud burst onwards.

Literature Cited

1. CEBAN, A. I., 1968: The content and accumulation of nucleic acids in the buds of the overwintering eyes of the vine during different stages of seasonal development. Fiziol. Rast. (Moskau) **15**, 329—335.
2. BONNER, J. and ZEEVART, J. A. D., 1962: DNA multiplication for expression of floral stimulus in *Pharbitis nil*. Plant Physiol. **37**, 296—304.
3. CHINOY, J. J., 1967: Studies in cellular and molecular aspects of floral induction and differentiation — A review. Indian J. Plant Physiol. **10** (2), 202—220.
4. GIFFORD, E. M. and TEPPER, H. B., 1962: Histo-chemical and autoradiographic studies of floral induction in *Chenopodium album*. Amer. J. Bot. **49**, 411—418.
5. JÁKÓ, N., 1970: Einfluß der Behandlung mit Adenin, Uracil und Tiba auf den Gehalt an Nukleinsäuren sowie an freien Aminosäuren der Rebenblätter. Mitt. Klosterneuburg **20**, 25—32.
6. KESSLER, B. R., BAK, R. and COHEN, A., 1959: Flowering in fruit trees and annual plants as affected by purines, pyrimidines and tri-iodo benzoic acid. Plant Physiol. **34**, 605—608.
7. MANIVEL, L., 1967: Effect of certain treatments on the fruitfulness of grape buds in Anab-e-Shahi variety. M. Sc. (Ag.) Diss. Univ. Madras, India.
8. NANAYA, K. A., 1966: Studies on fruit bud initiation and differentiation in some grape varieties of South India. M. Sc. (Ag.) Diss. Univ. Madras, India.

9. Nieman, R. H. and Poulsen, L. L., 1963: Spectro-photometric estimation of nucleic acid of plant leaves. Plant Physiol. **38**, 31—35.
10. Rajaram, S., Krishnamurthi, S. and Madhava Rao, V. N., 1965: Fruit bud initiation and differentiation in the grape (*Vitis vinifera* L.) variety Anab-e-Shahi. S. Indian Hort. (Coimbatore) **12**, 73—78.
11. Srinivasan, C., 1968: Effect of certain fertiliser treatments with reference to the time of application in grapes (*Vitis vinifera* L.) Var. Anab-e-Shahi. M. Sc. (Ag.) Diss. Univ. Madras, India.

Eingegangen am 22. 4. 1971

Prof. Dr. V. N. Madhava Rao
Division of Horticulture
Agricult. Coll. and Res. Inst.
Coimbatore 3
India