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Sugar, acid, and nitrogen in the developing berries of some grape varieties[1])

by

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Zucker, Säure und Stickstoff in den wachsenden Beeren einiger Rebsorten

Zusammenfassung. — Der Gehalt an Gesamtstickstoff, Gesamtsäure und Gesamtzucker in wachsenden Beeren wurde in regelmäßigen Abständen ermittelt. Dabei zeigte sich, daß der Gesamtstickstoff, auf das Trockengewicht bezogen, in der Zeitspanne zwischen 10 und 20 Tage nach der Anthese sehr schnell zunahm und danach bis zur vollen Beerenreife wieder absank. Es wird vermutet, daß der Höchstgehalt 20 Tage nach der Anthese mit der Phase eines maximalen Stickstoffbedarfes für den Aufbau der verschiedenen Gewebe zusammenfälllt.

Die Gesamtsäure in den wachsenden Beeren zeigte das bekannte Bild eines allmählichen Anstiegs während einer Dauer von 40 Tagen nach der Anthese sowie einer darauffolgenden allmählichen Abnahme bis zur vollen Reife der Beeren. Das Maximum fällt mit niedrigen Nachttemperaturen zusammen und zeigt eine stärkere Säuresynthese bei niedrigen Temperaturen an. Der allmähliche Säureabbau entspricht dem Anstieg der Tagestemperaturen und läßt eine Veratmung der Säuren bei hohen Temperaturen vermuten.

Die Zuckerakkumulation in wachsenden Beeren begann 50 Tage nach der Anthese. Die Akkumulationsrate war in der Zeitspanne zwischen 60 und 80 Tage nach der Anthese sehr hoch. Diese Periode fällt mit dem Beginn der dritten Phase des Beerenwachstums zusammen.

Introduction

The grape culture in Maharashtra State occupies a unique position in the Indian Union. Amongst the various commercial varieties of grapes grown in the State are the seeded varieties Bangalore Blue, Gulabi, Phakdi and Anab-e-Shahi. The exogenous application of various growth regulators to such varieties has not been very encouraging in improving the size of the berries or production of clusters. Since the development of seeded berries has been reported to be dependent on the native auxin and gibberellin status in the berry and seeds, it is important to study the various biochemical changes which affect berry growth and development. The present study follows the course of the total sugars, total acid and nitrogen.

Material and Methods

A composite sample of 25 g of berries was collected at 10-day intervals from anthesis until maturity to determine the total acidity in fresh grape berries of each variety. Five g of another sample were collected at the same time and were homogenized and transferred to 250 ml volumetric flask and the volume was made up by adding distilled water. This was then titrated against 0.1 N NaOH solution using phenolpthalein as an indicator. The acid content in the berries of each variety was calculated in terms of grams tartaric acid per 100 g of fresh berries.

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Total sugars were determined by the 'Lane and Eynon' method using methylene blue as indicator. For this purpose, 5 g fresh berries of each variety were crushed, homogenized and filtered. The volume of the filtrate was made up to 250 ml in volumetric flask by adding distilled water. Five ml each of Fehlings' A and B Solution were taken in a conical flask and the mixture was further diluted with distilled water. It was heated to boiling and the berry solution was allowed to run drop by drop from a burette till the solution in the flask turned brick-red which indicated the end point. From the sugar volume of standard solution, the percent of total sugars present in 100 g of the berries of each variety was calculated.

For an estimation of total nitrogen, 0—5 g of dried berries were used. Kjeldahl's method was adopted for the determination of nitrogen and is expressed as percentage dry weight basis.

Results and Discussion

Nitrogen

The total nitrogen content on dry weight basis during 1966—67, 1967—68 and 1968—69 are presented in Table 1.

During 1966—67 the N content in Bangalore Blue and Gulabi showed a sudden increase between 20 and 30 days after anthesis, followed by a rapid increase until 40 days and 50 days in Gulabi and Bangalore Blue respectively. After this there was a gradual decline in N in both varieties until maturity. In 1967—68 and 1968—69 the total N in all varieties showed a rapid increase between 10 to 20 days after anthesis, followed by a gradual decrease until maturity (in 1967—68) and a rapid decrease until 40 days after anthesis in all the varieties in 1968—69. There was further rise in the total N content in Gulabi between 50 and 60 days then it remained constant thereafter. The Bangalore Blue showed a gradual increase in total N until 90 days after anthesis. In Anab-e-Shahi, N content was found to decrease until 30 days after anthesis. Subsequently, intermittent increase in N content was recorded between 50—60 days and 70—80 days and remained almost constant thereafter. After October, pruning the resultant vegetative growth and production of inflorescences on the newly arising shoots is greatly dependent on the proper C/N balance within the vine. According to Nitsch (1951), the young growing points monopolize the nutrients, mainly nitrogen needed for growth from the adjacent tissues. During the early stage of berry development, when cell division is taking place at a rapid rate, the large quantity of protoplasm will accumulate large amounts of N. Accordingly, the increase in N content in the berries of all the varieties up to 20 days after anthesis appears to be due to greater accumulation of nitrogen in the new tissues. This observation is further supported by the findings of Hale and Weaver (1962) who reported that the cluster after berry set becomes a strong 'sink' into which photosynthates flow. Crafts (1961) has also shown that foods move from regions of synthesis to those of utilization. He has further pointed out that there is increasing evidence indicating a simultaneous co-related flow of many substances along the route from 'source' to 'sink' including foods, assimilates, sugars, growth regulators, viruses, etc. The declining trend in total N as berry growth progresses is in agreement with Dekaxos *et al.* (1966).

Sugars

The percentages of total sugars in the developing berries are presented in Table 2. The data reveal that the presence of sugars was not detected until 50 days after

Table 1

Changes in total nitrogen in developing grape berries (percent dry weight basis)

Veränderungen des Gesamtstickstoffgehaltes wachsender Beeren in Prozent des Trockengewichtes

Days after anthesis	1966—67		1967—68				1968—69			
	Bangalore Blue	Gulabi	Bangalore Blue	Gulabi	Anab-e-Shahi	Phakdi	Bangalore Blue	Gulabi	Anab-e-Shahi	Phakdi
At anthesis	0.40	0.51	0.45	0.45	0.45	0.46	0.35	0.52	0.52	0.51
10	0.45	0.49	0.49	0.42	0.40	0.45	0.41	0.62	0.35	0.62
20	0.50	0.54	2.42	2.21	1.64	2.54	3.41	1.98	1.70	3.60
30	2.35	2.50	1.95	1.92	1.35	1.42	1.60	1.54	1.65	2.61
40	1.80	1.75	1.83	1.80	1.07	1.34	0.69	0.77	1.50	0.58
50	1.75	0.90	1.27	1.11	1.27	1.35	0.90	0.80	0.89	0.69
60	1.20	1.11	1.22	0.98	1.35	1.25	0.86	1.82	1.70	0.91
70	1.30	1.20	0.88	0.80	0.98	0.97	1.12	1.71	0.89	0.74
80	0.90	0.85	0.75	0.92	0.51	0.72	1.21	1.78	1.72	1.71
90	0.99	0.70	0.87	0.89	0.48	0.42	1.72	1.61	1.24	1.31
100	0.60	0.90	0.52	0.70	0.50	0.54	1.40	1.66	1.71	1.70

Table 2

Total sugar content (%) and rate of increase of sugars in developing berries

Gesamtzuckergehalt (%) und Akkumulationsrate der Zucker in wachsenden Beeren

Days after anthesis	1966—67				1967—68								1968—69							
	Bangalore Blue		Gulabi		Bangalore Blue		Gulabi		Anab-e-Shahi		Phakdi		Bangalore Blue		Gulabi		Anab-e-Shahi		Phakdi	
	%	Rate	%	Rate	%	Rate	%	Rate	%	Rate	%	Rate	%	Rate	%	Rate	%	Rate	%	Rate
50	—	—	—	—	2.12	—	2.60	—	4.46	—	—	—	—	—	—	—	2.4	—	—	—
60	—	—	3.57	—	3.10	0.04	6.15	0.13	0.49	0.04	—	—	2.8	—	2.7	—	7.3	0.20	1.9	—
70	2.32	—	5.20	0.05	5.45	0.07	12.60	0.10	12.50	0.09	—	—	6.9	0.14	6.7	0.14	8.9	0.01	2.6	0.06
80	6.52	0.2	10.34	0.12	8.33	0.05	13.62	0.008	12.92	0.003	3.56	—	12.6	0.08	8.9	0.03	10.0	0.01	7.1	0.17
90	9.04	0.04	14.25	0.04	10.41	0.02	14.20	0.004	13.92	0.007	10.41	0.19	12.8	0.002	13.8	0.06	13.0	0.03	10.3	0.04
100	11.30	0.03	15.62	0.01	12.50	0.02	15.62	0.001	14.70	0.006	10.41	—	13.1	0.002	13.8	—	13.7	0.005	11.3	0.009

anthesis. In Bangalore Blue, the presence of a small quantity of sugar was observed 70, 50 and 60 days after anthesis during 1966—67, 1967—68 and 1968—69 respectively. This was followed by a very rapid increase in total sugars with maturity. The rate of increase was maximum after 70 days in 1967—68 and 1968—69 and, subsequently, the rate was seen to decrease. In Gulabi, the sugars were observed between 50—60 days after anthesis and increased rapidly thereafter, the rate of increase being maximum between 50—70 days in different years. In Anab-e-Shahi, the total sugar content showed a rapid increase between 50—70 days and the rate of increase slowed thereafter. In Phakdi, however, the total sugars in berries were detected 80 days after anthesis during 1967—68 and after 60 days in 1968—69. The rate of sugar increase was maximum between 60—80 days during 1968—69 and, subsequently, the rate decreased until maturity.

White (1907) has reported that rate of respiration of the young ovule is greatly stimulated after pollination and fertilization. For respiration the developing fruit requires sugar and organic acids and the resultant energy is utilized for building new tissues. Thereafter, there is no accumulation of sugars during the rapid growth period of the berry. Evidently, the observed absence of sugars in the berries of all varieties up to 50 days after anthesis appears to be the result of the continuous berry and seed growth recorded during this period. The available sugars have been utilized to provide the energy required for the growth processes leaving no sugar to accumulate in the berry. The presence of small quantities of sugars noticed in the berries after this period almost coincided with the beginning of third stage of berry development, during which the growth of berries was further reduced. It may also be pointed out here that the observed presence of the total sugars in the berries represents the balance left un-utilized out of the quantity either synthesized or translocated into the berry. Accordingly, the rapid increase in the sugar content recorded during the third stage of berry development, showed that there was an accumulation of sugars in berry either as the un-utilized balance or that translocated into the berry from the vine reserves. The prevailing low night temperatures during this period may also have favored the accumulation of sugars within that period also reported in a number of grape varieties in California by Bioletti *et al.* (1918) and Coombe (1960) which demonstrated that sugar and growth peaks of berry volume coincide during the second stage of berry growth. The second peak, according to Coombe may have been caused by the influx of sugars into the berry. In the present study, the second peak of berry volume also either coincided or followed the peak of sugar increase in all the varieties during all years. These findings are also supported by Coombe's hypothesis stated earlier. The observed rapid accumulation of sugars in the berry may also be explained on the basis of the theory of formation of a 'strong sink' in developing berries, advanced by Hale and Weaver (1967).

Organic acids

The changes in percentage titratable acidity in the berries recorded periodically from anthesis to maturity in Bangalore Blue and Gulabi varieties during 1966—67, 1967—68 and 1968—69 and in Anab-e-Shahi and Phakdi varieties during 1967—68 and 1968—69 are presented in Table 3. The acid content in the berries of all varieties increased gradually until 40—50 days, followed by a gradual decline until maturity. The percentage of total acidity recorded in Bangalore Blue, Gulabi and Anab-e-Shahi varieties gradually increased up to 30 days and showed subsequent gradual decrease until maturity of berries. In the berries of Phakdi variety, however, the

Table 3

Total acid contents per cent in terms of tartaric acid in developing grape berries

Gesamtsäuregehalt in Prozent, ausgedrückt als Weinsäure, in wachsenden Beeren

Days after anthesis	1966—67		1967—68				1968—69			
	Bangalore Blue	Gulabi	Bangalore Blue	Gulabi	Anab-e-Shahi	Phakdi	Bangalore Blue	Gulabi	Anab-e-Shahi	Phakdi
At anthesis	0.83	0.83	0.50	0.70	0.90	1.00	0.60	0.80	0.70	0.90
10	1.60	1.42	1.11	1.31	1.41	1.51	1.61	1.61	1.60	1.62
20	2.00	2.00	2.11	2.11	2.00	1.72	2.60	2.12	3.30	2.30
30	2.10	2.32	2.12	2.13	2.00	2.41	4.50	3.53	2.60	2.20
40	2.50	2.61	2.62	2.51	2.61	2.91	4.00	3.01	3.60	4.30
50	2.70	3.00	3.41	3.00	3.00	3.33	2.92	2.42	2.60	3.61
60	2.60	2.63	3.31	2.81	2.00	3.32	2.61	2.82	2.10	3.31
70	2.41	2.61	3.00	1.92	1.21	2.61	1.91	1.21	1.00	2.14
80	2.40	2.12	2.00	1.33	0.82	2.12	1.00	1.00	0.80	1.13
90	1.72	1.12	1.31	1.33	0.63	0.81	0.51	1.00	0.50	0.81
100	0.90	0.70	1.00	0.60	0.61	0.61	0.41	0.51	0.50	0.32

increase in percent total acid content was gradual to 30 days followed by a rapid increase up to 40 days and a gradual decline thereafter until maturity.

Total acid in the developing berries followed the familiar pattern of progressive increase, followed by a decrease in total acidity as observed also in the orange and apple. Total acid in the berries is influenced by the fluctuations in temperature. At temperatures above 30° C the malic and tartaric acid, which comprise almost 90 percent of the total acidity in grape berries, are reported to be respired (Bremond 1937). Similarly, low night temperatures favor synthesis and accumulation of organic acids (Nitsch 1953). The observed peaks of total acidity in the berries of all the varieties coincided with low night temperatures favorable for the greater synthesis of acids during this period. The rise in day temperatures recorded about 40 days after anthesis was accompanied by a decrease in the total acid, oxidation occurring as a result of prevailing high temperatures. The reduction in the total acid content in all the varieties as observed 40 days after anthesis is in agreement with the results of Iwohari *et al.* (1968) who also noticed a decline in total acids in seeded Tokay grape with the beginning of the third stage of berry growth.

Summary

Periodical data regarding changes in total nitrogen, total acid and total sugar of the developing berries revealed that the total nitrogen in dry weight basis increased very rapidly between 10—20 days after anthesis and decreased subsequently until maturity of berries. The maximum N content 20 days after anthesis is thought to coincide with the period of maximum requirement of N at this stage for building up of the various tissue.

The total acidity in developing berries showed the familiar pattern of gradual rise to 40 days after anthesis, followed by a gradual decline until maturity of berries. The maximum coincides with low night temperatures, indicating greater synthesis of acids at low temperatures. The gradual reduction of acidity until maturity corresponded with the rise in day temperature suggesting the respiration of acids at high temperatures.

The sugar accumulation in developing grape berries started 50 days after anthesis. The rate of accumulation was very high from 60—80 days after anthesis. This period coincides with the beginning of the third stage of berry growth.

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