Studies on the Translocation of Photosynthates in Satsuma Mandarin

III. Effect of Water Stress on the Metabolism of Sugars in the Fruit

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Summary

With decreasing moisture supply, the ^{14}C activity of the ethanol soluble fraction in the fruit increased in comparison with that of the ethanol insoluble fraction. Sugars may be major constituents of the former fraction, and polysaccharides those of the latter. It appears that the suppression of polysaccharide synthesis may result in more sugar accumulation in the juice. Although the translocation of photosynthates was depressed by withholding water, sugar content per fruit was rather higher than that of the controls. Thus, it was tentatively supposed that the hydrolysis of cell wall constituents may also occur leading to the high sugar content in the fruit under moisture stressed conditions.

Introduction

Although the effect of moisture stress on the growth of citrus fruit has been studied and discussed by many investigators, there are few approaches to determine the mechanism of sugar accumulation in the juice at high moisture tensions. The author's previous studies(4) postulated that besides the so-called concentration effect behaving in the sugar accumulation process in the juice of citrus fruit grown under water stressed conditions, some other factors should affect the sugar metabolism. It was also clarified that the rate of the translocation of photosynthates was decreased with lowering moisture supply(4). The presented study was undertaken to obtain direct information concerning the effect of water stress on the metabolism of sugars in the fruit.

Effect of water stress on the distribution of sugars at an early stage of fruit development

Materials and Methods

On April 5, 1971, 4-year-old trees of Okitsu

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Wase, an early ripening satsuma mandarin, were replanted into polyethylene pots 60 cm in depth and 50 cm in diameter. A granitic sandy soil was used for the potting. All the trees were fertilized with a 14-10-12 ($\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$) fertilizer at the rate of 50 g per pot. On August 2, all the leaves on a branch unit with 2 fruits each were allowed to photosynthesize under $^{14}\text{CO}_2$ for 1 hour. The soil moisture tension was kept within a range of pF 2.0 to 2.7. Twenty four hours after $^{14}\text{CO}_2$ feeding, the leaves were removed and differential treatments of soil moisture tension were made. For preparing water-stressed status, many holes 1 cm in diameter were drilled in the side of each pot so as to deprive the soil of water and pF was adjusted within a range of pF 3.2 to 3.5 throughout the experiment. Immediately after sampling, the fruits were weighed, and cut up into small pieces. They were extracted with 100 ml of boiling 80 percent ethanol. The mixture was simmered one hour on a steam bath and then centrifuged. After the supernatant liquid was decanted, the residue was re-extracted three times with ethanol. The supernatant liquid was transferred to a 1-liter flask. The ethanol was evaporated using a rotary evaporator and made up to 250 ml. A one ml

sample was withdrawn for the determination of radioactivity. The residue was dried to be weighed, and a 50 mg sample was subjected to combustion in order to evolve CO_2 quantitatively. The evolved CO_2 was trapped by an ethanolamine solution. The radioactivity of an aliquot was determined by a liquid scintillation spectrometer as described previously(4, 5).

Results

Tables 1 and 2 show the ^{14}C activity of ethanol soluble fraction and of ethanol insoluble fraction respectively. With increase of soil moisture stress, the radioactivity of the latter showed a marked decrease, whereas that of the former was kept high. Thus, when the moisture supply was suppressed, the ratio of ^{14}C activity of the ethanol soluble to that of insoluble fraction increased (Table 2). As for the plant supplied with sufficient amount of water, it may be well to say that at an early stage of fruit development, ethanol insoluble substances were actively synthesized and the photosynthates translocated to the fruit were utilized as major sources of sugar metabolism. Table 3 shows the effect of water stress on the sugar content of fruits. It is noticed that the water stress exerted on trees throughout the experiment caused an increase in sugar content.

Table 1. Activity of ^{14}C in the ethanol soluble fraction of fruit sampled on August 5.

Treatment	Fresh weight of fruit	^{14}C activity per fruit	^{14}C activity per gram fresh weight
	g	$\text{CPM} \times 10^4$	$\text{CPM} \times 10^4$
Water stressed*	16.14	264	16.4
Control	20.68	226	10.9

* Irrigated regularly until 24 hours after the $^{14}\text{CO}_2$ feeding, then, subjected to water stress; holes 1 cm in diameter were drilled in the side of each pot so as to deprive the soil of water and the pF was adjusted within a range of 3.2 to 3.5.

Table 2. Activity of ^{14}C in the ethanol insoluble fraction of fruit sampled on August 5.

Treatment	Dry weight of the residue	^{14}C activity per fruit	^{14}C activity per mg dry weight	Ratio** of ^{14}C activity
	mg	$\text{CPM} \times 10^4$	CPM	
Water stressed*	780	42.3	543	6.25
Control	1076	75.6	703	2.99

* See footnote of Table 1.

** The ratio of ^{14}C activity of the ethanol soluble to that of insoluble fraction.

Table 3. Effect of water stress on the sugar content of fruit.

Treatment	Reducing sugar		Total sugar	
	per fruit	per gram fresh weight	per fruit	per gram fresh weight
	g	mg	g	mg
Water stressed*	0.324	20.08	0.602	37.33
Control	0.297	14.38	0.575	27.82

* See footnote of Table 1.

Effect of water stress on the distribution of photosynthates at a maturing stage of fruit development

Materials and Methods

The experiment was started on October 22. Immediately after $^{14}\text{CO}_2$ feeding the following treatments of water supply were made.

Treatment 1: Soil was washed out from roots and the naked roots were exposed to the air during the experiment.

Treatment 2: pF value of the soil moisture was kept within a range of 2.7 to 3.3.

Treatment 3: pF value was kept from 2.5 to 2.2.

Extraction procedures and methods of the determination of radio carbon activity were the same as described above.

Results

Translocation of radio carbon was depressed and the total ^{14}C activity became low with increasing water stress. This was evident for both the ethanol soluble and ethanol insoluble

Table 4. Effect of low moisture supply on the translocation of photosynthates in the carpellary segment and rind at maturing stage of fruit (ethanol soluble fraction)*.

	Treat-ment**	Fresh weight	^{14}C activity per fruit	^{14}C activity per gram fresh weight
		g	$\text{CPM} \times 10^4$	$\text{CPM} \times 10^4$
Carpellary segments	1	80.14	150.3	1.87
	2	77.75	391.3	5.03
	3	104.70	599.1	5.72
Rind	1	15.85	109.9	6.92
	2	12.65	237.7	18.79
	3	16.99	309.4	18.19

* The experiment was started on October 22.

** Treatment 1. The roots were exposed to the air throughout the experiment.

Treatment 2. pF of the soil was kept from 2.7 to 3.3.

Treatment 3. pF of the soil was kept from 2.2 to 2.5.

Table 5. Effect of low moisture supply on the translocation of photosynthates and incorporation into the ethanol insoluble fraction of fruit.

	Treatment*	Dry weight of the residue ^g	¹⁴ C activity per fruit CPM × 10 ⁴	¹⁴ C activity per mg dry weight CPM	Ratio** of ¹⁴ C activity
Carpellary segments	1	1.16	2.72	16.3	55
	2	1.44	5.87	40.8	66
	3	2.34	13.77	58.8	43
Rind	1	3.16	5.79	18.3	19
	2	2.17	11.35	52.2	20
	3	2.75	26.28	95.3	12

* See footnote of Table 4.

** The ratio of ¹⁴C activity of the ethanol soluble to that of insoluble fraction.Table 6. The ratio of ¹⁴C activity of the carpellary segments to that of the rind.

Treatment*	The ratio in	
	ethanol soluble fraction	ethanol insoluble fraction
1	1.376	0.469
2	1.646	0.517
3	1.936	0.523

* See footnote of Table 4.

Table 7. Effect of low moisture supply on the total sugar content of fruit.

Treatment*	Total sugar content per fruit ^g	Total sugar content per gram fresh weight ^{mg}
1	9.50	118.5
2	8.75	112.5
3	8.00	76.4

* See footnote of Table 4.

fractions (Tables 4 and 5). The higher ratio of the former to the latter was shown in the higher moisture tension (Table 5). As shown in Table 6, the ratio of ¹⁴C activity of carpellary segments to that of the rind was higher in the controls than in the dry lot. This indicates that with increasing water supply, the translocation from the rind to the carpellary segments became more active. Total sugar content of the carpellary segments increased with increasing moisture tension. As to the sugar contents (Table 7), the more severe water stress resulted in the higher sugar content in the fruit. The results obtained in this experiment clearly pointed out that contrary to the decrease of ¹⁴C translocation, total sugar content per fruit was kept high in the water stressed condition.

Discussion

Generally, it may safely be said that the photosynthates translocated from leaves to fruit take part principally in a series of reactions of carbohydrate metabolism as sources of polysaccharide synthesis and respiratory substrates and also contribute to the accumulation of sugars in the juice. Accordingly, active translocation of photosynthates into fruit does not always play to increase the sugar content of the juice. Table 8 shows correlation coefficients between the fruit weight and sugar and/or acid content. Negative correlation was observed between acid and fruit weight, while no significant correlation between sugar content and fruit weight. In the previous paper(4), there should be postulated not only the concentration effect caused by moisture stress but also some other factors which were responsible for an increase of sugar accumulation. Thus, in this experiment, samples were extracted with ethanol to separate carbohydrates into ethanol soluble and insoluble fractions. In the ethanol soluble fraction sugars may be major components while most of the insoluble fraction are probably polysaccharides. As was already mentioned(3, 4), the ¹⁴C activity of ethanol soluble

Table 8. Correlation coefficients between fruit weight and sugar and/or acid content.

Date of sampling	Correlation coefficient	
	Fruit weight to sugar concentration	Fruit weight to acid concentration
October 21, 1966	0.107	-0.388*
November 20, 1966	0.211	-0.396*

* Significant at 5% level.

fraction increased with decreasing water supply. The present result agrees with that of PLAUT et al.(9,10) obtained with bean leaves. Thus, it is conceivable that at high moisture tension polysaccharide synthesis appears to be suppressed considerably so that sugars translocated into fruit fails to participate in the carbohydrate metabolism. This may result in more sugar accumulation in the juice. In the first experiment, differential treatments of water supply were made 24 hours after $^{14}\text{CO}_2$ feeding. Since most photosynthates of leaves were translocated during the first 24 hours after $^{14}\text{CO}_2$ feeding(6), it is conceivable that there was almost no difference in ^{14}C activity among the fruits. However, in the second experiment which was made at a maturing stage of fruit development there appeared significant differences between the treatments. That is, with increasing moisture tension, the translocation of sugars was severely suppressed. As for the second experiment, it should be noticed that the moisture control was started immediately after the $^{14}\text{CO}_2$ feeding. Further, it must be mentioned here that while the translocation of photosynthates was suppressed, sugar content per fruit was rather increased. Thus, it can be deduced that at high soil moisture tensions, not only the synthesis of polysaccharide such as hemicellulose was depressed but also some of the cell wall components were hydrolyzed. Generally it has been accepted that synthesized polysaccharides such as hemicellulose and pectic substances do not take part in the metabolism of sugars. Recently, however, LAMPORT et al.(7) have noticed the presence of cell wall enzymes which can hydrolyze those polysaccharides. WADA et al.(11) and MACHETT et al.(8) reported that enzymatic degradation of some hemicellulosic polysaccharides could lead to the loosening of cell wall matrix, which consequently induces expanding growth of cells. ESAU et al.(2) also noticed that a cell wall component may be digested during the process of cell growth. ASHIZAWA(1) reported that the thickness of the side wall of carpellary segments decreased at high moisture tensions. From this discussion, it appears that under water stressed conditions the degradation of cell wall constituents may occur leading to an increase of sugar content.

However, the difficulty is that the situations of carbohydrate metabolism at high tension is still a matter for conjecture. It remains to be further investigated.

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温州ミカンの光合成産物の転流および分配に関する研究（第3報）

水分供給の多少が果実内の糖の代謝におよぼす影響

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摘 要

1. 乾燥条件下におかれた果実では、アルコール可溶性分画中の ^{14}C 活性がアルコール不溶性分画中の ^{14}C 活性に比べて高い値を示した。アルコール可溶性分画の大部分は糖とみなされ、不溶性分画では多糖類が主体となつていると思われる。したがって、乾燥下で生長した果実が示す糖度の上昇は、単に水分の供給不足にもとづく物理的な濃縮効果によつて導かれるだけでなく、果実に転流して来た光合成産物から多糖類への生成系が抑制さ

れることもその一因と考えられる。

2. $^{14}\text{CO}_2$ 同化直後に乾燥処理を施すと、果実への光合成産物の転流が押えられたにもかかわらず、一果中の糖含量は対照区よりもむしろ高くなつた。このことから乾燥区では果実内の細胞壁構成多糖類が加水分解されて糖含量の上昇に寄与していることも推察される。乾燥にともなう光合成物質の代謝に関しては、なお類推の域にとどまることが多く、今後の追求が強く期待される。