CCLXIII. POLYPLOIDY AND VITAMIN C.

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(*Received November 2nd, 1933.*)

IN this communication, evidence is produced which lends strong support to the view that there exists a connection between the polyploid constitution of the plant and its vitamin C content. The experiments to be described were embarked upon as a consequence of some suggestive information which was obtained in a series of investigations on the apple. Some years ago it was observed by Zilva and his collaborators in the course of a comprehensive investigation on the influence of the physiological condition of the apple on its vitamin C content [Bracewell *et al.*, 1930, 1, 2] that the antiscorbutic potency of the Bramley's Seedling variety was very much greater than that of a number of other varieties examined. The striking nature of this observation was stressed by the writers at the time. Attention was then drawn by Prof. J. B. S. Haldane and later by Dr J. T. Bregger, Extension Horticulturist of the State College of Washington, Pullman, Washington, to the fact that the Bramley's Seedling was the only variety amongst those investigated which was a triploid (51 chromosomes in the somatic tissue), whilst the others were diploid varieties (34 chromosomes in the somatic tissue). Acting on this suggestion, Crane and Zilva [1931; 1932] made a comparative study of a number of triploid and diploid apples. They found that in both forms there was a variation in antiscorbutic activity amongst the varieties tested, but that the most active apples fell within the triploid category. The striking feature, however, was that certain triploid fruits showed as low a vitamin C content as some of the least active diploid apples and conversely that certain diploid varieties were as active, if not as the most active triploid apples, at least as some of the moderately active fruits of this category. In interpreting the results it was, therefore, difficult to arrive at a clear-cut conclusion. Crane and Zilva, nevertheless, considered the evidence as favouring the theory that a higher vitamin C content was associated with a higher number of chromosomes in the somatic tissue in the apple.

It is plain that conclusive evidence could only be obtained by comparing the polyploid and the diploid form from which the former was derived. Such comparison could not conveniently be made in the case of the apple. Crane and Zilva therefore suggested that fruits in which polyploidy could be induced with ease should form the future experimental material. In consequence the present authors chose the tomato as a starting-point.

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THE EXPERIMENTAL MATERIAL.

The normal tomato is a diploid with 24 somatic chromosomes, but it is found that when this form is decapitated, about 7 % of the adventitious shoots which arise in consequence of this treatment are tetraploids with 48 somatic chromosomes.

The contrasting characters used in this experiment were tall (*D*) and dwarf (*d*), red-fleshed fruit (*R*) and not red-fleshed (yellow) fruit (*r*). Through linkage phenomena the tall plants were also of the constitution *O* (round fruit), *P* (smooth fruit-skin) and the dwarfs were *o* (oval fruit) and *p* (rough skin).

The above diploid stocks of the genetic constitutions *DOPR*, *dopr*, *DOPr* and *dopR* were induced to form tetraploid shoots by the above method discovered by Winkler [1916] and developed by Jørgensen and Crane [1927]. Self-fertilisation of the diploid and tetraploid plants thus obtained therefore produced progeny of comparable constitutions in these four genetic categories, *i.e.* tall red, dwarf yellow, tall yellow and dwarf red. All these characters are known to be inherited according to Mendelian laws [*cf.* Lindstrom, 1925; 1932; McArthur, 1931]. The production of the experimental material is presented diagrammatically in Fig. 1.

Fig. 1. 1, Normal diploid ($2n$) tomato (24 chromosomes). 2, Diploid cut back at *a* giving rise to shoot *b* (diploid) and shoot *c* (tetraploid). 3, Diploid plant raised vegetatively from shoot *b* of 2. 4, Tetraploid plant raised vegetatively from shoot *c* of 2. Seeds from 3 and 4 supplied respectively diploid and tetraploid material for this investigation.

THE VITAMIN C TESTS.

The comparison of the vitamin C contents was carried out on the juice of the tomatoes, since the utilisation of the intact fruit might have yielded misleading information owing to variations in the juiceless pulp. Preliminary experiments carried out in 1932 revealed that in order to obtain truly comparable results several precautions had to be taken in carrying out the tests. One of the chief of these was against the instability of the vitamin in the juice. Very marked losses were recorded when the tomato juice was stored even at -20° for any length of time, a temperature at which the activity of many fruit tissues and juices is moderately stable. Further complications arising out of a difference in stability of the vitamin in juices originating from fruits of different constitutions could also not be excluded. As a result of several trials, the following procedure was finally adopted. The fruit was picked from plants growing in adjacent rows at Merton twice a week. The juice was prepared daily immediately before its administration to the experimental animals. The tomatoes were averaged as to size, as far as possible, and also as to degree of ripeness, before

being placed in a refrigerator at -20° the day previous to the preparation of the juice. To each 50 g. of the frozen tomato 20 g. of sand were added and the mixture finely ground. After squeezing through muslin, the residue was well ground again. The squeezed out juice was then added to the ground residue, ground once more and again pressed out through muslin. After centrifuging, the resulting yellow juice was administered to the animals without delay. A yield of juice equivalent to 80 % or more of the original weight of the tomato is thus obtained and the entire procedure does not take more than 30 minutes. The details of the biological test method have been given in previous communications.

Chemical data.

Owing to the limited quantity of experimental material at our disposal we were unable to examine it in great detail. This we hope to do when more material is available. We have nevertheless obtained a certain amount of information in this connection about the juices tested which has some bearing on this investigation.

H ion concentration. This was daily carried out colorimetrically, immediately after the preparation of the juice. There was little variation in the acidity of the juices. Those from diploid tomatoes showed a p_H value of 4.2-4.4, very exceptionally 4.6. The tetraploid juices were slightly more acid, about p_H 4.0. The acidity of both forms was probably due to the presence of weak organic acids since an appreciable quantity of ammonia was required to bring the solutions to p_H 7.0.

Indophenol-reducing capacity. As has been usual for a number of years in this laboratory, the juices were titrated daily with indophenol (dimethylamino-phenylindophenol) just before they were administered to the experimental animals. In these tomato juices, although it would be unsafe to generalise, there is some proportionality between the capacity of reducing this indicator and the antiscorbutic activity. It is therefore of interest to examine the titration results. Space does not permit citation of all the figures, but in Table I there are pre-

Table I. *Titration cc. N/1000 indophenol per cc. of juice.*

Tetraploids				Diploids			
<i>dRop</i>	<i>DROP</i>	<i>drop</i>	<i>DrOP</i>	<i>dRop</i>	<i>DROP</i>	<i>drop</i>	<i>DrOP</i>
3.3	3.2	3.1	3.2	2.5	2.3	2.8	2.0
4.1	4.5	3.5	4.5	1.7	1.6	2.4	1.9
4.5	4.8	4.6	4.9	2.0	2.0	2.7	2.0
4.8	4.9	4.5	4.5	1.6	1.7	1.9	1.6
4.1	5.3	4.7	4.7	1.7	2.6	2.6	2.3
4.2	4.7	4.3	4.1	2.0	2.3	2.6	2.6
3.9	3.0	3.4	3.1	2.9	3.3	3.4	3.2
Average for test:							
4.1	4.3	4.0	4.1	2.0	2.3	2.6	2.2

sented the weekly averages and the averages for the entire test. These figures are representative since there was little variation and consequently not much deviation from the average in the reducing capacity of the individual juices from day to day; extreme figures were obtained only rarely.

If it were assumed that the entire reducing capacity were due to ascorbic acid and that none of the "reversibly oxidised" form of the compound were present the tetraploids would contain 35 mg. and the diploids 20 mg. per 100 cc.

Fig. 2.

Fig. 3.

C. = chloroformed

+ = died

S. = scurvy

S.S. = slight scurvy

↓ = onset of clinical symptoms of scurvy.

V.S.S. = very slight scurvy

N. = normal

no S. = no scurvy

I. = intercurrent infection

Fig. 4.

Fig. 5.

C. = chloroformed

+ = died

S. = scurvy

S.S. = slight scurvy

↓ = onset of clinical symptoms of scurvy.

V.S.S. = very slight scurvy

N. = normal

no S. = no scurvy

I. = intercurrent infection

of juice. It will be seen later that the biological result was of a similar order in each case.

Dry matter. These estimations were not carried out daily but on several occasions during the progress of the test. The figures are given in Table II. The clear-cut nature of the results is obvious. The juices from the tetraploid varieties contained in every case more dry matter than those from the diploid varieties. It is hoped to continue the study of the chemical nature of the juices when more material is available.

Table II. *Dry matter* %.

Tetraploids				Diploids			
<i>dRop</i>	<i>DROP</i>	<i>drop</i>	<i>DrOP</i>	<i>dRop</i>	<i>DROP</i>	<i>drop</i>	<i>DrOP</i>
6.97	6.76	7.30	8.25	4.49	4.41	4.12	—
—	—	6.71	7.22	—	—	4.39	—
7.82	6.88	4.94	5.10	3.92	3.86	4.15	4.24
8.14	7.94	5.93	7.66	3.34	3.95	4.40	4.53
7.94	5.37	5.92	5.15	4.56	4.27	5.06	4.07
Average:							
7.72	6.74	6.16	6.67	4.08	4.12	4.42	4.28

Biological results.

These results are presented in Figs. 2, 3, 4 and 5 which give weight curves, clinical symptoms of scurvy and *post mortem* details. The results are straightforward and need hardly any comment. In every case the potency of juice from tetraploids is about twice as great as that from diploids and the activity in each case appears to be independent of the genetic characters of the varieties tested. The diploid variety *dopr* may be somewhat more active than the remaining diploid varieties. The corresponding tetraploid form, however, does not seem to possess a higher vitamin C content than the other tetraploids.

Interpreting the above results in terms of International Units and of ascorbic acid, the following data are obtained. In the case of the tetraploids, 1 cc. of juice gave rather better protection than is usually obtained with 1 cc. of decitrated lemon juice and was about equal in potency to 0.5 mg. of ascorbic acid [Hirst and Zilva, 1933]. It accordingly contains rather more than 1000 I.U. or about 50 mg. of ascorbic acid per 100 cc. of juice. The diploid juices on the same basis of assessment contain somewhat more than 500 I.U. or about 25 mg. of ascorbic acid per 100 cc. of juice.

It may be noted here that 1 mg. of ascorbic acid has always so far been observed to be rather more active than 2 cc. of the average decitrated lemon juice (20 I.U.) in this laboratory. As there is no significant variation in the response of the experimental animals in tests performed as above at different times, it may be safely assumed that this equivalence always holds true.

DISCUSSION OF RESULTS.

The results presented in this communication leave little doubt that a connection exists between polyploidy and vitamin C content in the tomato, which further strengthens the view of Crane and Zilva that a similar relationship exists also in the apple. This raises some interesting speculations concerning the latter fruit. It has already been mentioned that one of the diploids (Lane's Prince Albert) was as active as the moderately active triploid apples. Is it possible that the very active triploid apples were derived from a diploid strain or strains with a similarly high antiscorbutic activity? Conversely, is the origin

of the triploid Gravenstein with a very low vitamin C content to be traced to a diploid form with an exceptionally low antiscorbutic potency?

The genes investigated in this research seem to have no bearing on the vitamin C content of the tomato. It is not, however, excluded that other factors, whether genetic or not, may exercise such an influence. In fact we have obtained indications to this effect. For instance the wild tomato, *S. racemigerum*, which is a diploid shows a higher titration value with indophenol than do any of the tetraploid varieties of *L. esculentum* so far investigated. If the indophenol-reducing capacity should prove a reliable index of the antiscorbutic activity in this case, the higher vitamin content of this variety must be due to genes rather than the number of chromosomes.

The fruits from the tetraploid tomato plants are smaller than those from diploid plants but it is hardly likely that the higher concentration of vitamin C in the juice of tetraploids is indirectly due to the size of the fruit. We found that larger fruits showed if anything a higher indophenol-reducing capacity than the smaller ones belonging to the same plant and as was mentioned in this case the titration value was a fairly good indication of the antiscorbutic potency of the juice.

Reference must here be made to results recorded by Key [1933]. She examined one strain of each of a diploid and tetraploid tomato of comparable constitution for its vitamin C content by the tooth method and found no difference between their antiscorbutic activities. Her results show further that the vitamin C content of the above strains of tomato and of a sample bought in the open market was much lower than that recorded by us. We are unable to explain these divergencies.

SUMMARY.

The tetraploid and diploid forms of the strains of the tomato, *DOPR*, *dopr*, *dopr*, *DOPr*, obtained by the induction of polyploid method and subsequent vegetative propagation were examined for their vitamin C content by the prophylactic test. All the tetraploid strains were found to be about twice as active as the diploids. The tetraploids contained rather more than 1000 International Units or 50 mg. of ascorbic acid per 100 cc. of juice.

The above results strengthen the evidence obtained by Crane and Zilva that in the case of apples there is a connection between the vitamin C content and the number of chromosomes.

There is no association between the genetic factors *DR*, *dR*, *Dr* and *dr* and the vitamin C content of the tomato.

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