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HETEROAUXIN AND THE PRODUCTION OF TETRAPLOID SHOOTS BY THE CALLUS METHOD IN *BRASSICA OLERACEA*

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(With Five Text-figures)

1. INTRODUCTION

GREENLEAF (1938) obtained in *Nicotiana* hybrids a relatively high percentage of tetraploid callus shoots by applying a 1 % paste of heteroauxin to the cut surfaces of decapitated seedlings. It was not, however, possible to show that this high percentage (13·6) of tetraploids obtained was due to the heteroauxin treatment, since the simple decapitation technique does not produce calluses with shoots in *Nicotiana*.

In *Brassica oleracea* (kales and cabbages) calluses with leafy shoots can be obtained by simple decapitation, but a higher percentage of shoot-bearing calluses is obtained if heteroauxin paste is applied to the cut surface (Goldberg, 1938; Howard, 1938). In this paper it is shown that in *Brassica oleracea* heteroauxin treatment does not increase the percentage of tetraploid callus shoots over that obtained by simple decapitation, and there is also given a short account of the origin of callus shoots.

2. *BRASSICA OLERACEA* RESULTS

Most of the seedlings used for obtaining callus shoots were kales (see Table 1). The seedlings were decapitated when they had two large leaves. The decapitation was done as high as possible up the stem. Axillary buds were also removed at decapitation and as they appeared each week after decapitation. The decapitated plants were placed under bell-jars in a shady place so as to keep the cut surface moist. If the cut surface is allowed to become very dry, no callus shoots are obtained.

The heteroauxin (indole-3-acetic acid) treatment consisted in the application of a 1 % paste of the hormone in anhydrous lanoline to the cut surface immediately after decapitation. An account of the effects of this treatment, including the production of adventitious roots, will be found in both Goldberg (1938) and Howard (1938).

The callus shoots were separated from the callus when they were 2–4 in. tall and placed in moist sand in either a cold frame or under bell-jars. To obtain good rooting of these cuttings was not difficult. The most important factor in rooting them appeared to be to make the cut near the base of a leaf.

The cuttings were grown on to flowering. Diploids and tetraploids were then recognized by measuring pollen grains. Pollen-grain size was found to be a very satisfactory method of recognizing tetraploids in these experiments and also in colchicine work with *Brassica*. All cuttings except one eventually flowered. Some cuttings, however, missed flowering in their first two years, and it is this fact which accounts for this paper being written in 1941 and not in 1939. The tetraploids were confirmed by cytological examination and an account of their cytology has been published (Howard, 1939).

The results are given in Table 1. It will be seen that only three calluses produced tetraploid shoots. Nevertheless, the results do show that in *Brassica oleracea* heteroauxin treatment does not produce any striking increase in the percentage of tetraploid shoots obtained.

Table 1. *Frequency of tetraploid shoots from* Brassica oleracea *calluses*

Variety	Exp. nos.	Treatment	No. of plants de-capitated	No. of diploid cuttings	No. of tetra-ploid cuttings	% tetra-ploid
Cabbage	III, XII, XV	Decapitation only	35	74	0	0
1000-H Kale	XVII	Decapitation only	30	80	3*	3·7
1000-H Kale	XIX	Decapitation only	30	133	0	0
1000-H Kale	XXI	Decapitation only	10	28	0	0
	Total	Decapitation only	105	315	3	0·97
Marrow S. Kale	XXVII	1% paste heteroauxin	12	73	0	0
1000-H Kale	XXXI	1% paste heteroauxin	15	76	2†	2·6
	Total	1% paste heteroauxin	27	149	2	1·3

* All three cuttings (one of them was a diploid-tetraploid sectorial chimaera) came from the same callus, XVII 24; there were also seven diploid cuttings from this callus.

† Two cuttings from separate calluses, XXXI 7, seven diploid plus one tetraploid cuttings and XXXI 10, one diploid plus one tetraploid cutting. The tetraploid from XXXI 7 did not flower and its chromosome number was determined from root-tip counts.

3. Results in other plants

The percentages of tetraploid callus shoots obtained by other workers for various plants is given in Table 2. The results of Lindstrom & Koos appear to be exceptional. The *Brassica* results are similar to those shown in Table 1. It also appears that in *Lycopersicon esculentum* the frequency

of tetraploid shoots is about 6 % as compared with under 1 % in *Brassica* species. The 13·6 % of tetraploids obtained by Greenleaf might therefore be characteristic of *Nicotiana* and not due to the heteroauxin treatment.

Table 2. *Frequency of tetraploid shoots obtained by previous workers*

Species	Reference	Treatment other than decapitation	Total no. of cuttings	No. of tetraploid cuttings	% tetraploid
Lycopersicon esculentum	Jørgensen (1928)	None	278	16	5·8
Lycopersicon esculentum	Jørgensen (1928)	None	147	9	6·4
Solanum nigrum × *luteum* F_1	Jørgensen (1928)	None	342	7	2·0
Lycopersicon esculentum	Sansome (1930)	None	—	—	About 6
Lycopersicon esculentum	Lindstrom & Koos (1931)	Petrolatum	309	109	35·3
Brassica oleracea tetraploid × *carinata* F_1	Karpechenko (1937)	None	455	3	0·66
Brassica oleracea tetraploid × *chinensis* F_1	Karpechenko & Bogdanova (1937)	None	186	1	0·54
Nicotiana hybrids	Greenleaf (1938)	1 % heteroauxin	1973	270	13·6

4. THE ORIGIN OF CALLUS SHOOTS

Mather (1933) has given an account of the cytology of the origin of callus shoots in the tomato (*Lycopersicon esculentum*). He does not, however, describe the actual origin of the callus but only discusses the cytology of cells in the various parts of the callus. It is also extremely difficult to understand the plane in which the section drawn as Fig. 1 in his paper was cut. This is presumably due to the fact that Mather cut the calluses into small pieces for fixation.

Externally, tomato and *Brassica* calluses differ greatly in their development. In tomatoes the callus bulges out from the cut surface while in *Brassica* the cut surface remains flat and becomes slightly corky. Later in tomatoes shoots appear as bulges on the callus while in *Brassica* the shoot buds are first seen as small, usually purplish, tubercles pushing their way through the flat callus surface. Decapitated *Brassica* plants treated with heteroauxin produce bulgy calluses which resemble to a certain extent tomato calluses produced by decapitation alone.

Longitudinal sections through both tomato and *Brassica* calluses of different ages (1, 2 and 4 weeks after decapitation) were examined to ascertain the origin of calluses and callus buds. The short account of calluses given below agrees entirely with the very full accounts given by Priestley & Swingle (1929).

The first stage in the formation of a *Brassica* callus is shown in Fig. 1. The layer of cells, two or three cells down from the cut surface, becomes meristematic and divides by walls parallel to the cut surface.

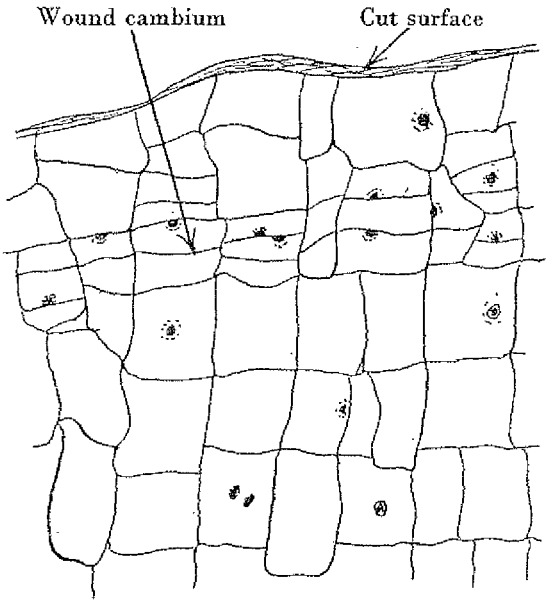

Fig. 1. Section through kale callus, 1 week after decapitation.

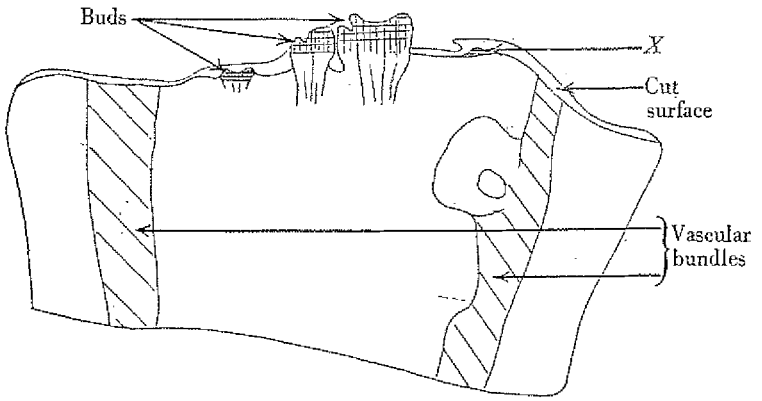

Fig. 2. Section through kale callus, 5 weeks after decapitation.

It is this layer of cells which becomes corky and protects the cut surface of the shoot. This layer can also be seen in Fig. 3, which is a section through an older kale callus. The meristematic tissue which will give rise to the callus buds also arises from this layer—this is shown quite well in

Fig. 3. The meristematic small-celled tissue is seen in Fig. 3 to be beginning to grow upwards and to be differentiating into a bud. The whole section (see Fig. 2) includes three recognizable buds in addition to the meristem which is marked *X*. As one removes shoots from a callus new buds are formed. These new buds are presumably formed from meristems like that marked *X* in Fig. 2. It will be noticed in Fig. 3 that the cells below the meristem have also divided.

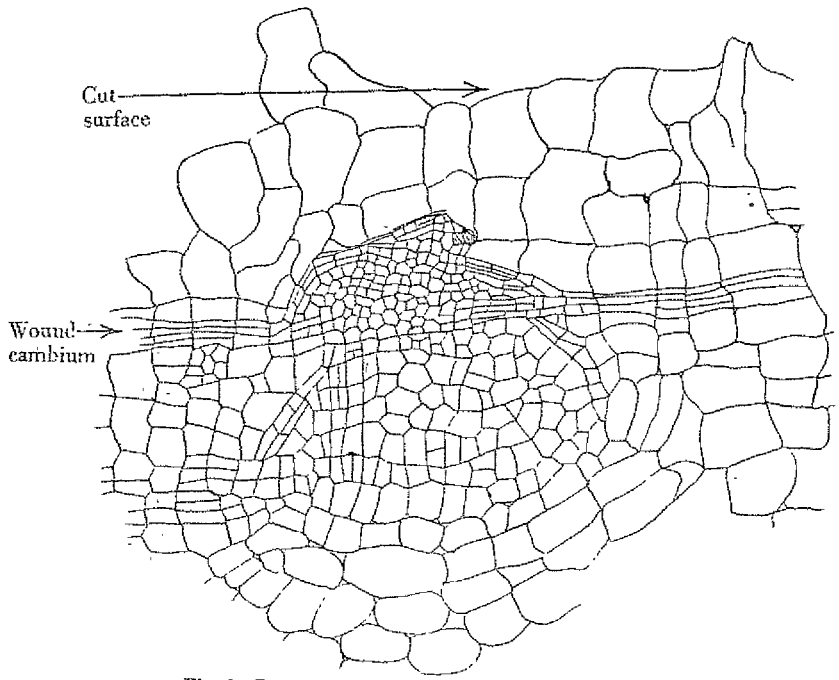

Fig. 3. Part of callus marked *X* in Fig. 2 enlarged.

Figs. 4 and 5 show a section through a one-week-old callus of a tomato. The cut surface of this callus had been kept moist by placing a bell-jar over the plant. It will be seen from Fig. 5 that no continuous wound cambium like that found in the *Brassica* calluses has been produced. Instead, there are two regions of small meristematic cells situated over the internal phloem of the vascular bundles. These meristematic regions grow rapidly and the adjacent tissues also become meristematic. It is the growth of such regions which produces the bulges of the callus.

In the sections of both the *Brassica* and tomato decapitated stems it can be seen that the cells, which divide to form the new meristematic tissues from which the callus buds later originate, are large vacuolated

ones. This is an important fact to be taken into account in considering the origin of tetraploid areas in these calluses. Figs. 1, 3 and 5 all illustrate this point. It can be seen that the cells adjacent to those which have produced the meristematic cells are large and vacuolated.

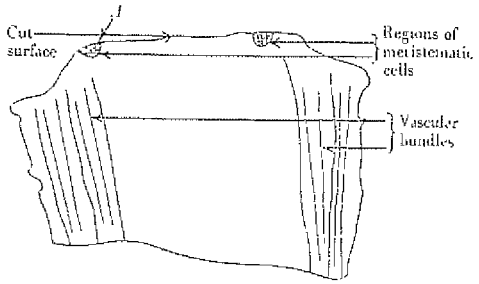

Fig. 4. Section through tomato callus, 1 week after decapitation.

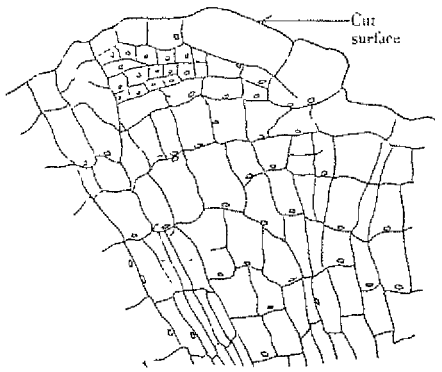

Fig. 5. Part of callus marked *A* in Fig. 4 enlarged.

5. Hypotheses to account for the occurrence of tetraploid callus shoots

It is possible to suggest four reasons why tetraploid callus shoots should be produced: (*a*) the occurrence of binucleate cells in stems, (*b*) the occurrence of tetraploid nuclei in some cells of the stem before decapitation, (*c*) the occasional failure of cell-wall formation when vacuolated cells divide, and (*d*) a breakdown in the normal course of cell division other than the failure of cell wall formation during the division of vacuolated cells.

Jorgensen (1928) supports a suggestion originally made by Winkler that the presence of binucleate cells in the stems before decapitation will account for the production of tetraploid shoots by calluses, and he refers to a paper by Beer & Arber (1919) on the occurrence of binucleate cells

in the pith and cortex especially of many plants. It is interesting to note that Beer & Arber did observe binucleate cells in *Brassica oleracea*. It is suggested that the two nuclei in the one cell divide at the same time and unite to form a single metaphase plate which will be tetraploid.

The second explanation—that tetraploid callus shoots are due to the presence of tetraploid nuclei in the stems before decapitation—appears to be highly probable. Thus Levan (1939) has shown that the old vacuolated cells in *Allium* roots contain diplochromosomes and that, when such cells are stimulated to divide by the application of heteroauxin, tetraploid metaphase plates are formed. The heteroauxin does not produce the tetraploid cells but only stimulates the old vacuolated cells which are already tetraploid to divide. Tetraploid cells are not produced in the young cells at the root tip. Similarly, Dermen (1941) found that polyploid cells were produced only in differentiated parenchyma tissues and not in the cambial regions of bean (*Phaseolus*) stems treated with naphthalene-acetic acid. Diplochromosomes and the subsequent formation of tetraploid metaphase plates in which the chromosomes are paired have been observed in many plants, e.g. *Spinacia* and *Cucumis* (references in Barber, 1940), in the nodules of Leguminosae (see Dermen, 1941), and in *Iberis semperflorens* (Figs. 14–16 of Manton, 1935). Since the meristems which ultimately give rise to callus buds are formed by the division of old vacuolated cells, the presence of tetraploid callus shoots would be expected from the results of Levan & Dermen. The differences in percentage tetraploids from calluses of *Brassica* and tomato have, however, still to be explained. This problem is considered in the last section of this paper.

The third explanation—that somatic doubling occurs in some cells of a callus because of occasional failure of cell-wall formation when large vacuolated cells divide—was suggested by Mather (1933). He gives a number of figures showing cell divisions in large vacuolated cells and others showing binucleate cells. Mather did not ascertain the precise stage at which nuclear fusion takes place in the binucleate cells but agrees with the suggestion of Lindstrom & Koos that it may well occur at several points in the nuclear cycle. While it is quite reasonable to suggest that cell-wall formation may occasionally fail when vacuolated cells divide, it is important to note that Sinnot & Bloch (1941) have shown that there is a regular mechanism by means of which division of the nucleus in vacuolated cells is followed by cell-wall formation. It seems unlikely that this mechanism would break down in 6% of the cells in the tomato calluses.

Fourthly, tetraploid cells might be produced in callus formation by a breakdown in the normal processes of cell division other than failure of cell-wall formation, e.g. a breakdown in anaphase separation followed by the formation of a single tetraploid resting nucleus. Such a breakdown might be due either to the cells being vacuolated or to the effect of wound hormones.

While it is not possible to decide conclusively what is the reason for the occurrence of tetraploid callus shoots, the second explanation appears to be the most probable. It ought, however, to be possible to observe the first divisions of the vacuolated cells in decapitated tomato stems and ascertain if tetraploid metaphases with paired chromosomes occur.

6. Heteroauxin and the production of tetraploids

It has previously been mentioned that the work of Levan (1939) showed that heteroauxin did not produce tetraploid cells but only stimulated cells which were already tetraploid to divide. We should not, therefore, expect to obtain a higher percentage of tetraploid shoots from heteroauxin treatment than from calluses obtained by decapitation only. This was found to be true for *Brassica oleracea*.

Assuming that tetraploid cells in calluses are produced by the division of vacuolated cells which contain nuclei with diplochromosomes, it would appear from the percentages of tetraploid callus shoots obtained that less than 1 % of the vacuolated cells in kale stems contain nuclei with diplochromosomes, while the corresponding frequencies in tomato and *Nicotiana* are 6 and 13 % respectively. An alternative explanation of the low frequency of tetraploids obtained in *Brassica* is that diploid cells might grow much faster than tetraploids in the calluses. Observations of diploid-tetraploid chimaeras produced by colchicine treatment in *Brassica* do not support this suggestion.

7. Summary

1. In *Brassica oleracea* the frequency of tetraploid callus shoots obtained by decapitation only was found to be about 1 %. The frequency from calluses obtained by heteroauxin treatment was also about 1 %.

2. The origin of new meristems from vacuolated cells in calluses is described.

3. Theories to account for the occurrence of tetraploid callus shoots are considered. It is suggested that the tetraploid areas in calluses are caused by the division of vacuolated cells which contain nuclei with diplochromosomes.

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