INDUCTION OF MITOSIS BY SUCROSE IN EXCISED AND ATTACHED DORMANT BUDS OF SUNFLOWER (*HELIANTHUS ANNUUS* L.)

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Summary

The mitotic frequency in dormant cotyledonary buds of light-grown sunflower (*Helianthus annuus* L.) plants is low. It may be sharply reduced, either by floating detached buds on a mineral solution for 6–24 hr, or by darkening plants for 3 days. This lowered mitotic frequency can be increased by the supply of sucrose alone, either to detached buds via the flotation medium, or to attached buds via the hypocotyl.

Decapitation above the cotyledons induces intense mitotic activity in the axillary buds of light-grown plants 16–24 hr later; but not in those of darkened plants.

We suggest that a bud's activity, and hence, in some cases, its dormancy status, may be affected by its carbohydrate level.

I. INTRODUCTION

Bud activity, particularly release from dormancy, is commonly assessed by measurements of lengths of the enlarging buds or resultant shoots. These measurements may therefore not be fully informative about any event which initiated activity and which occurred some days previously. Information bearing more closely on such an initiating event would be secured by investigating an earlier stage of activity, and an objective of the present work was to enquire whether observations on mitotic frequency could be used for this purpose.

In many plants the dormant buds axillary to cotyledons provide suitable material, because mitotic activity in them is either absent, or reduced to a low level. Moreover, such buds may readily be forced into mitotic activity by removal of the top above them, and this is rapidly succeeded by visible growth (Wildman 1957).

II. MATERIALS AND METHODS

(*a*) *Plants*

Sunflower (*Helianthus annuus* L.) plants were grown from two seed samples, one of commercial origin, the other being an inbred line. Both samples gave essentially similar results. Plants were grown under natural illumination in a temperature-controlled glasshouse providing a day–night alternation of 25°C/20°C, and were used when the internode above the cotyledons had virtually ceased extension.

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(*b*) *Buds and Bud Cultures*

Buds were exposed for severance from plants with a minimum of injury by peeling back the cotyledons. Buds were variable in size and in development, as judged by the number and size of leaf primordia present. However, except for the epidermis and at the most two leaf traces, the cells were relatively undifferentiated and largely non-vacuolated. The nuclei occupied a large part of the cell volumes.

Detached buds were floated on autoclaved solutions of varied compositions. When cultures containing sucrose were to be maintained longer than 6 hr, bacterial and yeast growth was suppressed by the addition of 7 p.p.m. each of penicillin and streptomycin. Except for brief periods during excision and while changing solutions, the cultures were kept in darkness and at 25°C.

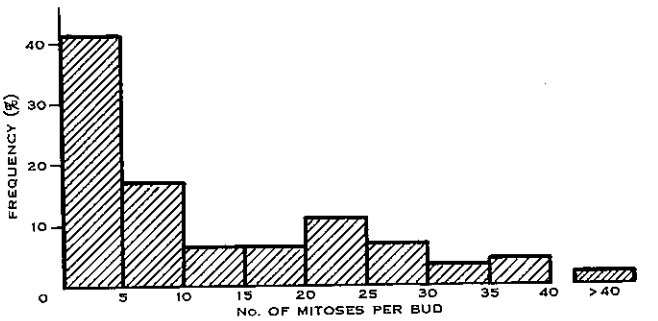

Fig. 1.—Frequency distribution of number of mitoses per dormant bud. Buds fixed immediately on excision. Based on 140 buds.

(*c*) *Mitoses*

At the time of excision, or at the end of treatment, the buds were fixed in solutions of ethanol–glacial acetic acid (3:1 v/v), softened briefly in 1N HCl at room temperature, stained with acid lacmoid, and smeared so that essentially all the cells could be examined. A mitosis was scored only when the chromosomes were fully evident. Total numbers of mitoses per bud were usually estimated by surveying alternate transects of each bud, it having been established that this procedure of observing one-half of the total cells in a bud gave a valid estimate. About 20 buds were examined from each treatment.

Results are presented as mean number of mitoses per bud. Significances were established by the Kolmogorov–Smirnov two-sample test which examines frequency distributions (Siegel 1956). Because of the possibility of undue weighting, in any sample, by one or a few buds with an abnormally high number of mitoses, examination of the means is a less rigorous test of significance between treatments than examination of frequency distributions.

III. Results

(*a*) *Frequency of Mitotic Figures Found in Dormant and in Active Buds*

In the intact sunflower plant the cotyledonary buds very rarely, if ever, grow to macroscopic dimensions, whereas, when the plant is decapitated, the buds become macroscopic 4–5 days thereafter.

The mitotic frequency found in dormant buds was variable, both within and between samples. Results from the examination of 140 dormant buds from six experiments extending over a period of 7 months are plotted in Figure 1. All samples were taken during the morning hours. While an occasional bud was found to have more than 40 mitotic figures, 58% of the buds had less than 11, and 83% less than 26 figures. There appeared to be no close correlation between bud size and number of mitoses, nor is there necessarily a close correspondence of mitotic activity in the two buds from the same plant. We did not examine in detail the rise and fall in mitotic frequency which accompanies the early growth of the bud, and the subsequent onset of dormancy. However, once the dormant state had been achieved, no correlation was discerned between mitotic frequency and either the length of stem or number of internodes above the cotyledons, up to the time the cotyledons withered.

TABLE 1

NUMBER AND FREQUENCY DISTRIBUTION OF MITOSES IN BUDS FROM INTACT AND DECAPITATED LIGHT-GROWN AND DARK-GROWN PLANTS

Plant Treatment	Mean No. of Mitoses per Bud	Percentage of Buds with following Numbers of Mitoses:					
		0	1–5	6–10	11–50	51–100	>100
Light-grown plants							
Intact	5·5	26	42	5	26	0	0
48 hr after decapitation	>200	0	31*		0	18	51
Dark-grown plants							
Intact	0·5	85	13	2	0	0	0
48 hr after decapitation	0·2	90	10	0	0	0	0

* Denotes percentage number for both classes combined.

In contrast to this situation with dormant buds, decapitation of light-grown plants at the internode between the cotyledonary and first leaf nodes resulted in an increase in the frequency of mitoses per bud which could be detected as early as 16 hr after decapitation. By 48 hr there were some hundreds of mitoses per bud (Table 1).

(b) Effect on Mitotic Frequency of Floating Detached Dormant Buds on Solutions

If bud inhibition in an intact plant arose as a result of accumulation of a substance such as auxin, or a specific diffusible inhibitor, excision of buds into solutions might be expected both to interrupt the supply and to lower the endogenous level by leaching, and thus lead to enhanced mitotic activity. Such an expectation was not realized. When detached dormant buds were floated on one-quarter strength Hoagland's solution, the number of mitotic figures did not rise but declined continuously until virtually no mitoses were apparent after 24–48 hr. By the end of 6 hr the numbers had fallen to about one-half those present at the time of detachment. However, when sucrose was a constituent of the flotation medium the decline was not observed, but rather the numbers were greater than at the time of detachment, as is indicated by the

distributions of Figures 2(*a*) and 2(*b*), in which the results of several experiments are pooled.

In some of these experiments constituents other than sucrose were present in the medium. A more rigorous demonstration of the action of sucrose was provided in the following way. Detached buds were floated on Hoagland's solution for 6 hr, at which time a sample was fixed (the controls of Table 2), and another sample was transferred to Hoagland's solution containing 0·2% sucrose, and then fixed after a further 24 hr. This method had the advantage of lowering the mitotic activity in buds to be

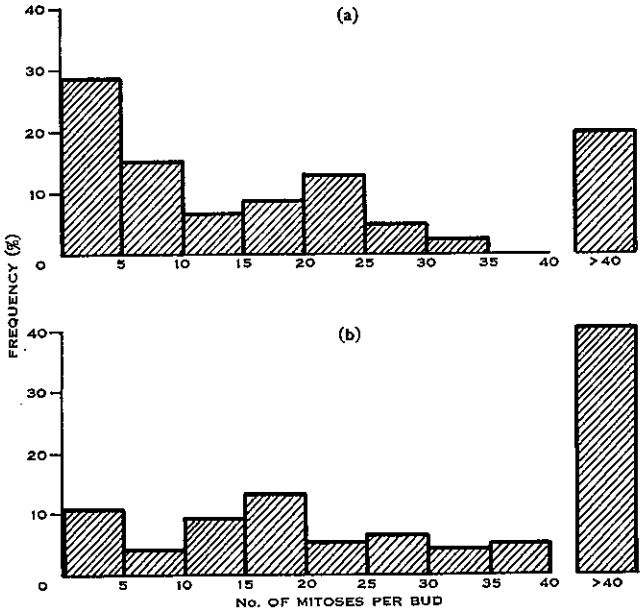

Fig. 2.—Frequency distributions of numbers of mitoses per bud after sucrose treatment. (*a*) Buds fixed after 2·8 hr incubation on a medium containing sucrose. Based on 45 buds. (*b*) Buds fixed after 24 hr incubation on a medium containing sucrose. Based on 74 buds.

treated with sucrose. At the same time, the comparison provides a minimal estimate of sucrose action since, as noted above, the decline in mitotic activity of buds on Hoagland's solution alone would have continued even further in the following 24 hr.

The results for four experiments are collected in Table 2. In all cases the average number of mitoses per bud was increased by sucrose treatment.

From estimates of mitotic frequencies alone, inferences may not properly be drawn about treatment effects on the number of cells undergoing division, except in the limiting case when the frequency associated with one treatment or condition is zero, and this is compared with another treatment for which there is a positive finite frequency. In the above short-term experiments no gross growth of the buds occurred, so that alterations in the proportions of various types of tissues containing cells capable of division are unlikely to have occurred. One may infer, therefore, that sucrose has

either promoted new mitoses, or affected the time course of the mitotic cycle in such a way that the cytologically apparent phase was retarded relative to the concealed phase. The close approach of many of the control values to zero, together with the magnitude of the increase in frequency in the treated buds, render the former explanation more probable.

(c) *Effect on Mitotic Frequency of Applying Sucrose to Attached Dormant Buds*

The above findings raise the question whether a change in the carbohydrate status of intact plants could be correlated with a change in the frequency of mitoses found in their dormant buds. When the carbohydrates were depleted by holding plants in the dark for 3 days, the average number of mitoses per bud was much

TABLE 2

EFFECT OF SUCROSE ON MITOTIC ACTIVITY IN DETACHED BUDS

Control buds fixed after 6 hr on Hoagland's solution, and treated buds after a further 24 hr on sucrose solution. Significance of differences calculated from Kolmogorov–Smirnov two-sample test

Expt. No.	Antibiotic Concn.* (p.p.m.)	Mean No. of Mitoses per Bud		Significance of Difference
		Control	Sucrose (0·2%)	
1	0	8·5	15·5	$P < 0·05$
2	20	0·0	7·3	$P < 0·01$
3	7	1·9	3·7	$P < 0·05$
4	7	3·2	16·2	$P < 0·01$

* Each of penicillin and streptomycin.

reduced. Moreover, decapitation of the dark-treated plants entirely failed to promote mitotic activity by the end of 48 hr—a result strikingly at variance with that from decapitation of light-grown plants (Table 1).

The effect on the mitotic activity of buds on plants which were supplied sucrose after a 3-day dark treatment was next examined. In experiment 1 of Table 3 control buds were excised and fixed at the same time as the plants from another sample were severed in the hypocotyl about 5 cm below the cotyledons, and the cut ends placed in a sucrose solution. After 12 hr in the dark, buds from the sucrose-treated plants were excised and fixed. In experiment 2 of Table 3, the sucrose-treated plants were treated similarly. In this case the controls were also treated similarly except that the cut shoots were placed in water. The results show that the supply of sucrose engendered a significant rise in mitotic activity in the short span of 12 hr.

IV. Discussion

Some simple carbon source is universally required in media appropriate for the long-term culture of various tissues. It is therefore not unexpected that sucrose should be required for the culture of excised buds, though in these experiments the preparations were maintained for only a matter of hours. Of more interest is the fact that mitoses could be initiated in the dormant detached buds by the sole addition of sucrose to the medium.

It has been amply demonstrated for animal tissues that (1) mitosis is an energy-requiring process; (2) the energy requirement is primarily in the antephase — that period immediately preceding prophase; (3) once a cell is in a competent state. division may proceed independently of further external energy supply. The evidence on these

Table 3

Effect of sucrose on mitotic activity in attached buds on dark-grown plants

Sucrose solutions supplied via hypocotyl for 12 hr. In experiment 1 control buds were excised and fixed at the same time as treated shoots were transferred to sucrose. In experiment 2 control buds were on shoots supplied with water instead of sucrose. Significance of difference from control calculated from the Kolmogorov–Smirnov two-sample test

Expt. No.	Mean No. of Mitoses per Bud			
	Control	Sucrose (0·2%)	Sucrose (0·8%)	Sucrose (1·6%)
1	0·8	5·4*	—	—
2	1·3	2·6	10·8**	10·1**

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

points generally has been reviewed by Bullough (1952) who thus regards mitosis as an all-or-none process. However, the situation is much less documented for plant tissues. Brown and Rickless (1949) have shown, indirectly by observing cell numbers, the stimulating effect of sucrose on cell division in excised root tips, and Wilson and collaborators (Wilson 1959), by direct observation of mitoses in excised root tips, have shown the ability of sugars and related compounds to restore competence to cells which have lost it during a brief period of starvation. The present results thus confirm these findings in another type of excised tissue.

The plant growth regulators 3-indolylacetic acid (IAA), gibberellic acid, and kinetin have been shown to induce mitosis, both in excised tissues and in intact plants (Das, Patau, and Skoog 1956; Sachs, Bretz, and Lang 1959*a*, 1959*b*; Haber and

Luippold 1960*a*; 1960*b*; Haber 1962). Where this has occurred, it must be considered either that they have acted on cells competent in the sense defined by Bullough (1952), or that, in addition to any other function they have, they are also concerned with the mobilization of energy-rich substrates to specific sites. Such a function has been found for some animal hormones.

The results from attached buds support the suggestion that mitotic activity may be used as a satisfactory index of bud activity at a stage earlier than is usually attempted. They also show that lack of substrates may be a primary limitation of mitotic activity in certain regions of intact plants.

Two main types of theory have been advanced in connection with bud dormancy and apical dominance:

(1) *Nutritive theories*—in which the inactivity is assumed to be due to the absence of an essential nutrient (Loeb 1924; Gregory and Veale 1957).

(2) *Inhibition theories*—in which IAA (Thimann and Skoog 1934) or another specific diffusible substance inhibits activity (Snow 1937, 1938; Hemberg 1949, 1958; Phillips and Wareing 1958).

These two types of theory have been linked by suggestions that IAA diverts an assumed limiting factor from buds, which thus remain dormant. Went's (1939) caline theory is a special case of this (for reviews see Thimann 1939; Gregory and Veale 1957). Several facts indicate that a bud's activity may be controlled by its carbohydrate status, and thus provide support for the first type of theory. These are (1) mitotic activity observed in dormant buds varies daily and seasonally; (2) lowering the carbohydrate status, either in the attached condition by darkening, or in the detached condition by culturing on a carbon-free medium, markedly lowers the frequency of mitoses; (3) decapitation of darkened, carbohydrate-depleted plants fails entirely to provide mitotic activity.

On the other hand, findings that IAA can be transported acropetally into lateral buds, and that the degree of inhibition of growth of buds, following IAA application via the stems, is proportional to the bud IAA content, constitute strong evidence that IAA is causally involved in apical dominance (Wickson and Thimann 1958, 1960). These two viewpoints need not necessarily conflict if it could be envisaged that IAA controls the transport or leakage of carbohydrate into axillary buds. A similar view has recently been put forward by Booth *et al.* (1962) who conclude "It would seem that auxin-directed transport may be important in the redistribution of nutrient reserves . . ., and may also play a part in apical dominance and the correlative inhibition of buds."

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