Plant Physiol. (1977) 60, 282-285

Cytokinin as a Possible Component of the Floral Stimulus in *Sinapis alba*

Received for publication December 3, 1976 and in revised form April 7, 1977

GEORGES BERNIER, JEAN-MARIE KINET, ANNIE JACQMARD, ANDREE HAVELANGE, AND MONIQUE BODSON

Laboratoire de Physiologie Végétale and Centre de Physiologie Végétale Appliquée (IRSIA), Département de Botanique, Université de Liège, Sart Tilman, B4000-Liege, Belgium

ABSTRACT

Results of previous investigations indicated that one of the early and essential events occurring in the apical meristem of *Sinapis alba* L. during the transition to flowering is the release to mitosis of the G_2 nuclei; the trigger to mitosis is generated in the leaves and its movement out of the leaves begins around 16 hours after the start of the inductive treatment. The mitotic wave in the meristem culminates 10 hours later.

In this paper, it is shown that a single application of a cytokinin (benzyladenine or zeatin) at concentrations ranging from 1 to 20 μg/ml directly to the apical bud of vegetative plants, at a time corresponding to the time of movement of the mitotic trigger in induced plants, produces a mitotic wave which is very similar to that found in induced plants. It is thus proposed that the mitotic component of the floral stimulus in *Sinapis* is a cytokinin. As the cytokinins are completely unable to induce flowering, it appears that there is a multicomponent floral stimulus in this species.

The physiology of flowering has been dominated for 40 years by the concept that there is one substance—called the *floral hormone* or the *floral stimulus*—which specifically evokes flowering and which is common to all higher plants (7, 17). All attempts to isolate this stimulus have failed and its chemical nature is still completely unknown (28).

Since the direct approach which consists in trying to extract, purify, and characterize the stimulus has yielded very little information so far, we thought it worthwhile to try an indirect approach, *i.e.* to try to gain insight into its nature from the kind of early changes that it produces at the shoot apical meristem.

The plant used in this study was *Sinapis alba*, a long day species which can be induced to flower by a single long day. One of the first detectable changes in the apical meristem of *Sinapis* during the transition from the vegetative to the reproductive condition is the release to mitosis of a large population of G_2 nuclei (6, 12). This early mitotic wave always culminates 26 to 30 hr after the start of the inductive treatment.

Further studies have shown that it was impossible to dissociate the flowering process at the meristem of *Sinapis* from this mitotic activation (5), and that a similar early mitotic rise occurred in the evoked meristems of all species so far examined in this respect (13, 16, 25, 27). We concluded that the early mitotic wave was an essential component of floral evocation.

By subjecting vegetative plants of *Sinapis* to a single day of 11 or 12 hr, it was however possible to induce this mitotic event in the absence of flowering (5). This possibility of fractional evocation in *Sinapis* suggested that the floral stimulus may consist of more than one active component and that the early mitoses are triggered by a specific component, called the mitotic component.

Other supporting evidence for a multicomponent floral stimulus was supplied by defoliation experiments which showed that the patterns of movement out of the leaves of the mitotic component and of the slowest moving component of the floral stimulus were not similar in some environmental conditions (3).

Here, our aim was to obtain information on the chemical nature of the mitotic component of the floral stimulus in *Sinapis*.

MATERIALS AND METHODS

The plant used was *Sinapis alba* L. The growing techniques were described elsewhere (5). The plants were maintained in the vegetative condition by growing them in 8-hr short days. These plants were referred to as the "control plants." When 65 days old, some plants were treated with various chemicals (detailed later). Aqueous solutions of the chemicals were always applied to a small cotton plug carefully inserted between the young leaves of the apical bud. Four hr after the start of each treatment, a small amount of solution was added to keep the cotton plug moist during a few additional hr. A total amount of about 0.3 ml was applied/bud. Tween-20, at a concentration of 0.1%, was added to all solutions. Preliminary experiments have shown that a 0.1% Tween solution had no effect on mitotic activity in the apical meristem. Treatments of plants during a dark period were carried out in weak green safelight.

Some 65-day-old plants were induced to flower by exposure to one 20-hr long day, and then returned to short days. These plants will be referred to as the "induced plants." Other plants of the same age were subjected to one 12-hr critical day. Flowering in the different experimental batches was assessed by examining the apical meristems of 15 plants under a dissecting microscope 2 weeks after the start of the treatments (5).

To examine changes in the percentage of cells undergoing mitosis at the meristem, apical buds were fixed in Navashin's fixative and the sections were stained with Ehrlich's hematoxylin. Interphase nuclei and mitoses were recorded in the median longisection of each meristem. Six meristems were used in each experimental batch.

DNA synthesis in the apical meristem was investigated by use of ^{3}H-labeled thymidine (5 μCi/plant; specific radioactivity: 5 Ci/mmol; labeling time: 4 hr) and autoradiography of histological sections as described elsewhere (6). The labeled and unlabeled nuclei were recorded in the median longisection of each meristem. Six meristems were used in each experimental batch.

Unless stated otherwise, the percentages of mitoses and labeled nuclei were determined in a meristematic area including both the central and the peripheral zones of the meristem.

All of the experiments were repeated once or twice. Per cents of mitoses and of labeled nuclei were compared by the χ^2 test.

RESULTS

Effect of Various Compounds on Mitotic Activity in the Apical Meristem. According to the literature, a stimulation of cell divisions in plant tissues can be obtained following the action of a variety of natural compounds, such as sucrose (1, 18, 26), gibberellins (4, 11, 22), cytokinins (8, 14, 20), and cAMP (19). All of these compounds could then be considered as possible candidates for the mitotic component of the floral stimulus in *Sinapis*.

It was known from previous experiments (3) that, in induced plants, the movement of the mitotic component out of the leaves always began around 16 hr after the start of the inductive treatment. Within the apical meristem, the early mitotic wave culminated 10 hr later, *i.e.* at 26 hr (6).

In a preliminary experiment, the chemicals listed above were individually applied to the apical buds of plants continuously kept in short days. The application was made at a time corresponding to the time of movement of the mitotic component in induced plants, *i.e.* at the middle of the 16-hr long night (Fig. 1). The apical buds of treated plants were collected 10 hr later, *i.e.* at a time corresponding to the early mitotic peak in meristems of induced plants. These buds were used for the determination of the mitotic index within the apical meristem.

We found (Fig. 2) that 2% sucrose and GA_3 at 25 μg/ml were without any effect on mitotic activity. Cyclic AMP at a concentration of 36 μg/ml markedly depressed the mitotic index. Only the cytokinins, BA or Z,[1] respectively, at 5 and 20 μg/ml could bring about a stimulation of mitotic activity similar to that recorded in the meristems of induced plants. Among the tested chemicals, only cytokinins were thus kept for further studies.

Kinetic Study of the Mitotic Activation Produced by the Cytokinins. Apical buds of plants kept in short days were treated with BA or Z (Fig. 1), but these buds were collected in the present experiment at various times after the treatment (Fig. 3). BA and Z at all tested concentrations produced a rapid increase in mitotic activity, starting at 6 hr and culminating at 10 to 14 hr. With BA at 10 μg/ml, the mitotic index remained high after 18 hr. At lower concentrations of BA (5 and 1 μg/ml), the mitotic index decreased after 14 hr, but rose again after 18 hr and reached a second peak at 30 hr. With Z, the mitotic index generally dropped after the first peak, the transient and weak increase at 18 to 22 hr not being significant.

When the different zones of the meristem were considered separately, the stimulation of mitotic activity in response to the cytokinin treatment occurred everywhere within the meristem (Figs. 4 and 5). The rise of the mitotic index was first detected in the peripheral zone (at 6 hr) and was apparent in the central zone and in the pith-rib meristem 4 hr later. This result was found with either BA or Z, and at all tested concentrations.

Cytokinin Effect on DNA Synthesis. DNA synthesis was investigated in meristems of plants kept in short days and treated as shown in Figure 1 with BA 10 μg/ml or Z 1 μg/ml (Fig. 6). A slight but significant increase in DNA duplication was recorded in meristems treated with BA, the stimulation began 14 hr after the start of the application and remained relatively constant until the end of the experiment. Separate examination of the different zones indicated that the rise in DNA synthesis was clearly apparent only in the peripheral zone, with very weak increase, if any, in the central zone and no increase at all in the pith-rib meristem. With Z there was no rise at all of DNA synthesis.

Cytokinin Effect on Flowering. BA or Z applied once at various concentrations up to 100 μg/ml to the apical buds of plants kept in short days (see Fig. 1) never induced flowering. They were also completely unable to induce flowering if applied at low concentrations (up to 20 μg/ml) but for several days.

[1] Abbreviation: Z: zeatin.

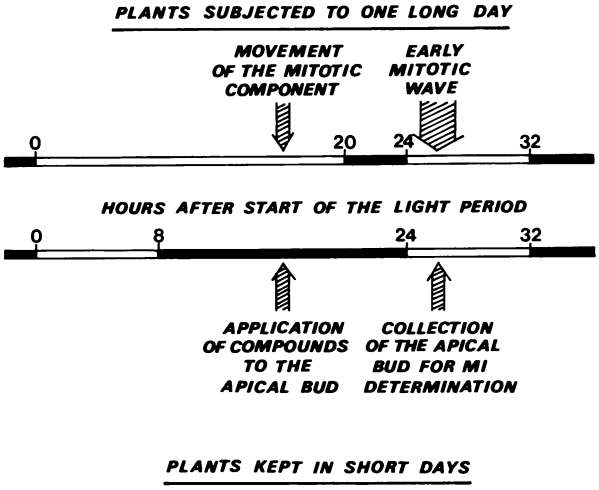

FIG. 1. Scheme of experiment designed to select the chemicals which can produce a mitotic stimulation similar to that induced by the long day. Explanation in the text. MI: mitotic index.

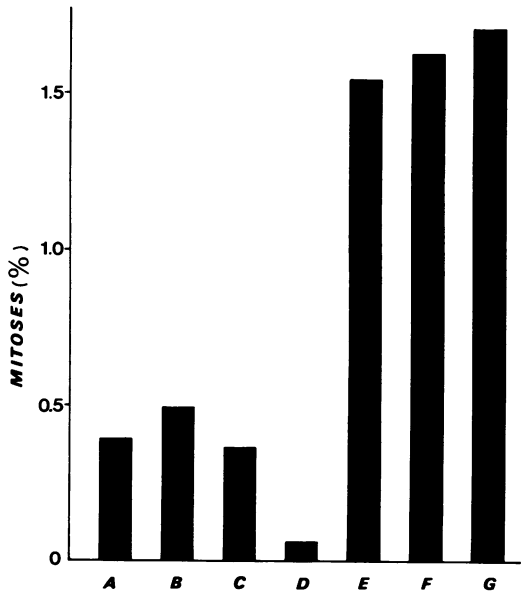

FIG. 2. Mitotic index in the apical meristem of plants kept in short days, 10 hr after application of water (A); 2% sucrose (B); 25 μg/ml GA_3 (C); 36 μg/ml cAMP (D); 5 μg/ml BA (E); or 20 μg/ml Z (F). In G, mitotic index in the meristem of plants induced to flower by one 20-hr day, and collected 26 hr after the start of the long day.

DISCUSSION

Cytokinins are generally considered to be plant cell division factors. Since the work of Skoog and Miller (24), they were known to elicit cell proliferation in cultured plant tissues, but there was far less direct evidence until now that they fulfill the same function in intact plants, particularly in shoot apical meristems (15). The results presented here clearly indicate that in intact *Sinapis* plants these compounds perform the same role as in tissue culture. The effect of cytokinins on mitotic activity is very specific since it is not observed with the other chemicals which were tested. The physiological nature of the mitotic stimulation is shown by the fact that it is achieved by very low amounts of exogenous cytokinins since most of the 0.3 ml of solution applied per bud must remain in the cotton plug and only a small (but unknown) part reaches the apical meristem.

The stimulation of mitotic activity produced by BA always extends for a longer period than the stimulation produced by Z

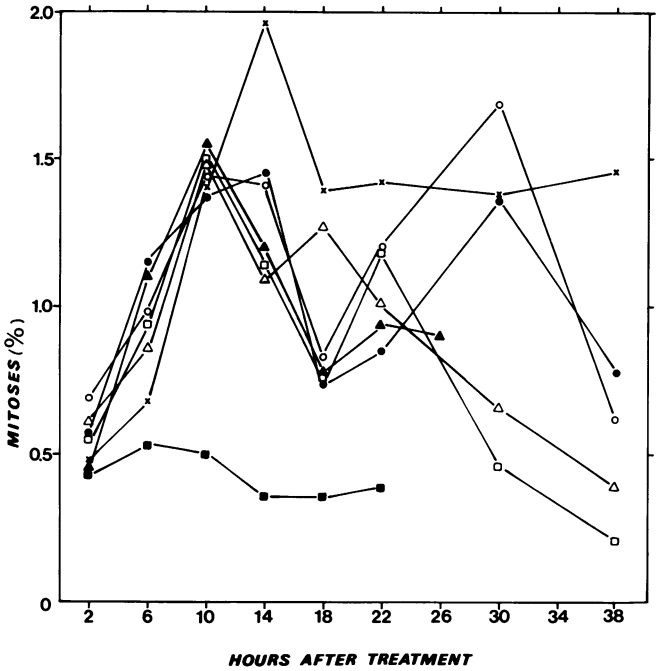

FIG. 3. Mitotic index in the apical meristem of plants kept in short days, at various times after application of 10 μg/ml BA (×); 5 μg/ml BA (○); 1 μg/ml BA (●); 20 μg/ml Z (△); 10 μg/ml Z (▲); 1 μg/ml Z (□); or water (■). Results averaged from two to three experiments.

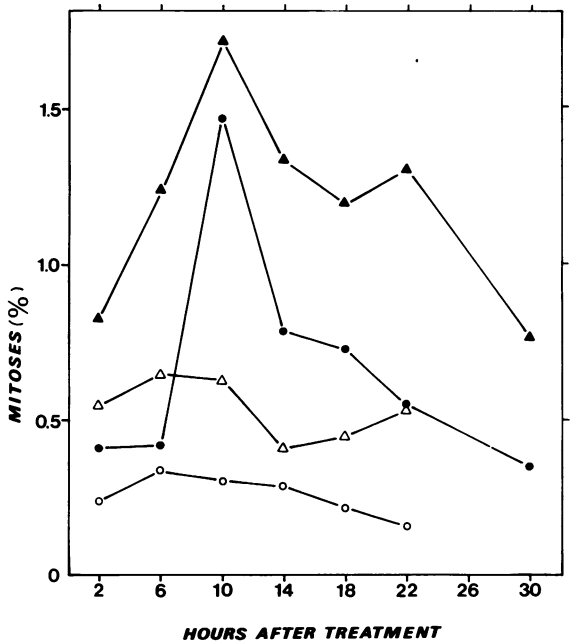

FIG. 4. Mitotic index in the peripheral (△, ▲) and central (○, ●) zones of the apical meristem of plants kept in short days, at various times after application of 20 μg/ml Z (▲, ●), or water (△, ○). Results of an individual experiment.

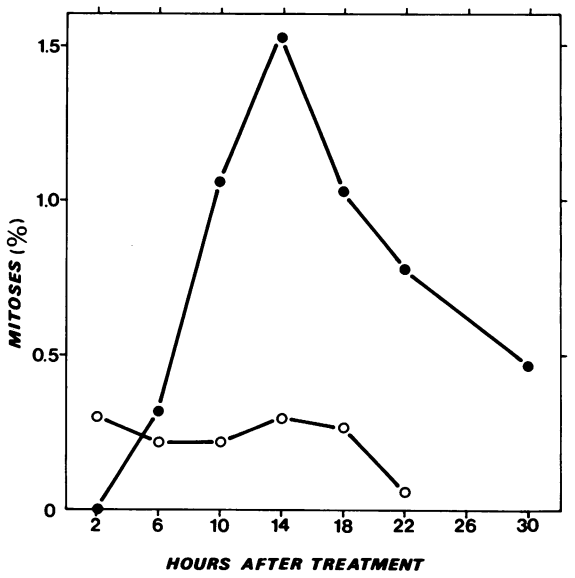

FIG. 5. Mitotic index in the pith-rib meristem of plants kept in short days, at various times after application of 1 μg/ml BA (●), or water (○). Results of an individual experiment.

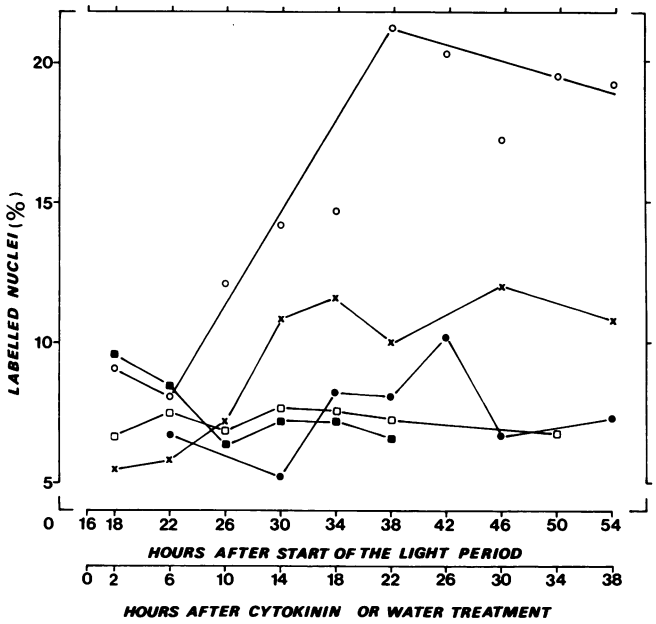

FIG. 6. DNA synthesis in the apical meristem of plants kept in short days, at various times after application of 10 μg/ml BA (×); 1 μg/ml Z (□); or water (■). For comparison, DNA synthesis in the apical meristem of plants subjected to one 20-hr day (○), or to one 12-hr day (●) has been included. The figure is constructed in such a way that the time of cytokinin application to plants kept in short days coincides with the time of movement of the mitotic component of the floral stimulus in plants receiving a 20- or a 12-hr day.

(Fig. 3), suggesting that the synthetic cytokinin BA persists longer in the tissues than the natural cytokinin Z because it is probably less rapidly metabolized.

The changes in time of the mitotic index after a treatment with a cytokinin have been compared to those occurring after the application of a 20-hr long day or a 12-hr critical day (Fig. 7). Obviously, the mitotic stimulation produced by Z at 1 μg/ml is very similar to that induced by a 12-hr day, and that by BA at 10 μg/ml is very similar to that induced by a 20-hr day. These results strongly suggest that the early mitotic activation that occurs in the meristems of plants subjected to a long day or to a critical day is produced by a cytokinin or that the mitotic component of the floral stimulus in *Sinapis* is a cytokinin.

This proposition is reinforced when a comparison is made of the results obtained for the different zones of the meristem after a cytokinin treatment and after application of a 20-hr day (6). In both cases, the stimulation of mitotic activity in the peripheral zone is 4 hr earlier than in the central zone and the pith-rib meristem.

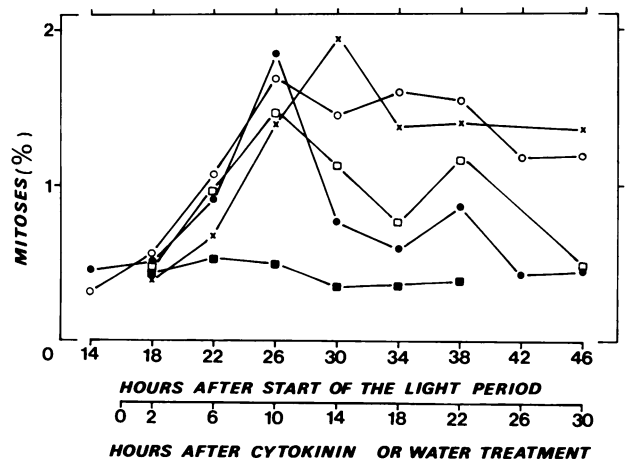

FIG. 7. Mitotic index in the apical meristem of plants kept in short days, at various times after application of 10 μg/ml BA (×); 1 μg/ml Z (□); or water (■). For comparison, mitotic index in the apical meristem of plants subjected to one 20-hr day (○), or to one 12-hr day (●) has been included. The figure is constructed as Figure 6.

In the meristems of plants induced to flower by a 20-hr day, the early mitotic wave is followed by a dramatic rise in DNA synthesis peaking at 38 hr (Fig. 6) (6). This is not so in the meristems of vegetative plants treated with a cytokinin. In several other experimental systems, it has also been shown that cell division is far more sensitive to the cytokinin level than DNA synthesis (8, 14, 20, 23). The conclusion is that the dramatic rise in DNA synthesis, which occurs in the meristems of induced plants and which has been clearly associated with the production of flower primordia (6), is controlled by something other than a cytokinin.

The suggestion that there is another component in the floral stimulus in this species is also strongly supported by the observation that the cytokinins are completely unable to induce flowering in *Sinapis*.

If a cytokinin is the mitotic component of the floral stimulus it must be produced in the mature induced leaves and translocated from there to the apical meristem. Evidence for foliar production and export of cytokinin is scarce in the literature, although some data suggest that such a possibility is by no means excluded (2, 9, 10, 21). Experiments are now in progress in our laboratory to test this possibility in *Sinapis*. Alternatively, a cytokinin may not be a component of the floral stimulus but its production could be enhanced within the apical meristem by the arrival from the leaves of a mitotic component of different and unknown nature.

Acknowledgment—We wish to thank G. Sylvestre for technical assistance in these experiments.

LITERATURE CITED

1. BALLARD LAT, SG WILDMAN 1964. Induction of mitosis by sucrose in excised and attached dormant buds of sunflower (*Helianthus annuus* L.) Aust J Biol Sci 17:36-43
2. BENNINK GJH 1974 Flower development in *Chrysanthemum* under long-day conditions by injections with the cytokinin benzyladenine. *In* Plant Growth Substances 1973. Hirokawa Publ Co, Tokyo pp 974-979
3. BERNIER G, M BODSON, JM KINET, A JACQMARD, A HAVELANGE 1974 The nature of the floral stimulus in mustard. *In* Plant Growth Substances 1973. Hirokawa Publ Co, Tokyo pp 980-986
4. BERNIER G, R BRONCHART, A JACQMARD, G SYLVESTRE 1967 Acide gibbérellique et morphogenèse caulinaire. Bull Soc R Bot Belg 100: 51-71
5. BERNIER G, JM KINET, M BODSON, Y ROUMA, A JACQMARD 1974 Experimental studies on the mitotic activity of the shoot apical meristem and its relation to floral evocation and morphogenesis in *Sinapis alba*. Bot Gaz 135: 345-352
6. BERNIER G, JM KINET, R BRONCHART 1967 Cellular events at the meristem during floral induction in *Sinapis alba* L. Physiol Vég 5: 311-324
7. CHAILAKHYAN MK 1937 Concerning the hormonal nature of plant development processes. CR Acad Sci URSS 16: 227-230
8. FOSKET DE, SHORT KC 1973 The role of cytokinin in the regulation of growth, DNA synthesis and cell proliferation in cultured soybean tissues (*Glycine max* var. *Biloxi*). Physiol Plant 28: 14-23
9. HEIDE OM 1972 The role of cytokinin in regeneration processes. *In* H Kaldewey, and Y Vardar, eds, Hormonal Regulation in Plant Growth and Development. Verlag Chemie, Weinheim pp 207-219
10. HEWETT EW, PF WAREING 1973 Cytokinins in *Populus* × *robusta* (Schneid): light effects on endogenous levels. Planta 114: 119-129
11. JACQMARD A 1968 Early effect of gibberellic acid on mitotic activity and DNA synthesis in the apical bud of *Rudbeckia bicolor*. Physiol Vég 6: 409-416
12. JACQMARD A., JP MIKSCHE, G BERNIER 1972 Quantitative study of nucleic acids and proteins in the shoot apex of *Sinapis alba* during transition from the vegetative to the reproductive condition. Am J Bot 59: 714-721
13. JACQMARD A, MVS RAJU, J, KINET, G BERNIER 1976 The early action of the floral stimulus on mitotic activity and DNA synthesis in the apical meristem of *Xanthium strumarium*. Am J Bot 63: 166-174
14. JOUANNEAU JP N TANDEAU DE MARSAC 1973 Stepwise effects of cytokinin activity and DNA synthesis upon mitotic cycle events in partially synchronized tobacco cells. Exp Cell Res 77: 167-174
15. KENDE H 1971 The cytokinins. Int Rev Cytol 31: 301-338
16. KING RW 1972 Timing in *Chenopodium rubrum* of export of the floral stimulus from the cotyledons and its action at the shoot apex. Can J Bot 50: 697-702
17. LANG A 1965 Physiology of flower formation. *In* W. Ruhland, ed, Encyclopedia of Plant Physiology Vol 15. Springer-Verlag, Berlin pp 1380-1536
18. LANGENAUER HD, EL DAVIS, PL WEBSTER 1974 Quiescent cell populations in apical meristems of *Helianthus annuus*. Can J Bot 52: 2195-2201
19. LUNDEEN CV, HN WOOD, AC BRAUN 1973 Intercellular levels of cyclic nucleotides during cell enlargement and cell division in excised tobacco pith tissues. Differentiation 1: 255-260
20. PATAU K, NK DAS 1961 The regulation of DNA synthesis and mitosis in tobacco pith tissue cultured *in vitro*. Chromosoma 11: 553-572
21. PHILLIPS DA, CF CLELAND 1972 Cytokinin activity from the phloem sap of *Xanthium strumarium*. Planta 102: 173-178
22. SACHS RM, CF BRETZ, A LANG 1959 Shoot histogenesis: the early effects of gibberellin upon stem elongation in two rosette plants. Am J Bot 46: 376-384
23. SIMARD A 1971 Initiation of DNA synthesis by kinetin and experimental factors in tobacco pith tissues *in vitro*. Can J Bot 49: 1541-1549
24. SKOOG F, CO MILLER 1957 Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp Soc Exp Biol 11: 118-130
25. TAILLANDIER J 1971 Evolution de l'activité mitotique et des synthèses d'ADN dans le point végétatif de l'*Anagallis arvensis*, au cours de l'eupériode inductive et des deux premières dyspériodes qui la suivent. CR Acad Sci 272: 219-222
26. VAN'T HOF J, CJ KOVACS 1972 Mitotic cycle regulation in the meristem of cultured roots: the principal control point hypothesis. *In* MW Miller, CC Kuehnert, eds, The Dynamics of Meristem Cell Populations. Plenum Press, New York pp 15-32
27. WADA K 1968 Studies on the flower initiation in *Pharbitis* seedlings, with special reference to the early stimulation of cell division at the flower-induced shoot apex. Bot Mag 81: 46-47
28. ZEEVAART JAD 1976 Physiology of flower formation. Annu Rev Plant Physiol 27: 321-348