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Certain Mitotic Effects of Kinetin, Gibberellic Acid, Maleic Hydrazide, and Indoleacetic Acid in Onion Roots

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Abstract. Growing, intact, adventitious roots from the bulbs of *Allium cepa* were treated with 1 ppm and 10 ppm solutions of kinetin, gibberellic acid, maleic hydrazide, and indoleacetic acid, and with paired combinations of these. The effect on the rate of mitosis was studied by counting the mitotic figures present in the distal 5 mm. of the root tip in serial longitudinal sections. Roots treated with kinetin alone or in any combination showed marked inhibition of mitosis. There was initial inhibition followed by an after-effect of stimulation of mitosis in roots treated with gibberellic acid, with indoleacetic acid, or with a combinaton of these. Maleic hydrazide did not reverse the action of any of the auxin-like substances tested, but the after-effect of stimulation was not seen when it was present. Roots treated with maleic hydrazide contained many cells in which the chromosomes showed aberrations including fragmentation and changes which gave the chromosomes a beaded appearance.

Although the problem of the mechanism of auxin action remains unsolved, it is evident that auxin relations in roots must differ greatly from those in shoots. After reviewing the literature on the action of auxin in the root, Torrey (1956) and Aberg (1957) pointed out the need for further study of effects at the cellular level.

Most available data for the action of auxin on roots pertain to effects on the total elongation of the organ, to which both cell division and cell elongation contribute. Progress toward an understanding of fundamental mechanisms of action requires that a distinction be made between effects on these two processes. The present investigation is a study of effects on cell division only of certain growth regulating substances on the root tip of *Allium cepa.*

Four growth regulating substances were used: kinetin (K), which stimulates cell division under certain circumstances; gibberellic acid (GA), which has certain auxin-like characteristics; indoleacetic acid (IAA), the type auxin with which others must be compared; and maleic hydrazide (MH), a growth inhibitor which in certain test reactions appears to reverse the action of auxin.[1]

Intact onion roots were treated with each of these substances alone and combined in all possible pairs. Effects on cell division were studied as reflected in numbers and kinds of mitotic figures

[1]The kinetin was graciously supplied by Professor Folke Skoog of Wisconsin University; the gibberellic acid, by Dr. E. F. Alder of Eli Lilly and Company; and the maleic hydrazide, by Dr. J. W. Zukel of the United States Rubber Company.

present in serial longitudinal sections of the meristematic region of the root tips.

Materials and Methods

Bulbs of common white onion were placed over flasks containing tap water until a large number of roots about an inch long had developed. Bulbs selected for uniformity, with roots of about equal diameter, length, and apparent vigor, were then transferred to flasks containing the various dilutions of the chemicals.

Solution was accomplished with about 2 ml. ethyl alcohol and dilution was with unbuffered tap water, to avoid inducing cytological abnormalities which sometimes result from the use of buffers (Kaufmann and Das, 1955). This allowed a pH fluctuation between 6.5 and 7.2 excepting for the IAA solutions, which dropped to 4.5.

At about 11 a.m. each day, five roots were taken from each treatment solution and from the control. The distal 5 mm. of the root tips were fixed immediately, with aspiration, in Randolph's Craf solution, and dehydrated and embedded by the standard tertiary butyl-paraffin method. Each root tip was serially sectioned longitudinally at 10μ and stained with Flemming's triple stain.

In four alternate (non-adjacent) sections at the center of each serial, all metaphases, anaphases, and telophases present in all tissues of the entire section were counted. The counts were averaged, and the figures used in the tables thus represent the means of twenty counts from five roots.

The entire series of experimental treatments was carried out three times.

Results

Table 1 shows the results obtained in one experimental series. Figures for the other two series compare very well. In every case, the experiments were continued for as long as a sufficient number of living roots remained. Roots treated with 1 ppm dilutions remained alive, but all had been removed for sectioning at 7 days. Roots treated with the 10 ppm dilutions had lost their turgidity (the common criterion of death) after 3 days.

Discussion

Effects of Kinetin

In the experiments reported here, kinetin-treated roots showed a marked inhibition of mitosis, from which they had not recovered at 7 days.

Guttman (1956) investigated the effect of kinetin on cell division in intact onion roots, and by a statistical study of counts

Table 1
Mean Numbers of Mitotic Figures Present Per 10 μ Central Section of Roots Treated With Growth Regulating Substances

Days	Control	K	GA	IAA	MH	K+GA	K+IAA	K+MH	GA+IAA	GA+MH	IAA+MH
						1 ppm					
1	17.1	3.1	10.8	5.8	7.6	2.9	0.8	6.1	7.6	7.2	3.7
2	16.5	5.0	14.2	9.0	10.1	3.9	4.8	9.1	8.5	5.8	6.4
3	18.6	1.5	14.6	15.4	5.2	5.2	5.6	6.5	7.8	7.7	3.8
4	18.8	2.8	18.5	24.6	2.0	8.1	6.8	7.2	14.8	6.8	6.0
5	19.8	7.9	26.9	27.7	2.2	8.9	7.3	6.1	21.8	8.1	5.0
7	17.0	6.4	18.3	19.9	1.8	13.8	1.1	1.6	26.0	1.6	0
						10 ppm					
1	17.4	5.1	14.7	5.6	4.1	4.5	3.8	8.3	3.5	3.2	3.5
2	16.0	2.3	7.0	0.7	3.7	1.1	1.4	2.7	2.0	1.3	0
3	17.5	0.2	2.4	0.1	0	0	1.6	0.5	0.2	1.3	0

made on random fields of squash preparations reached the conclusion that it increases the rate of mitosis. In the present study, the counts were made on sectional material, and the results are in agreement with the chemical findings of Jensen (1958) which also indicate an inhibition of cell division by kinetin in the intact root of onion.

Effects of Gibberellic Acid

GA has recently been shown to stimulate cell division in shoots (Sachs and Lang, 1957; Bradley and Crane, 1957; Greulach and Haesloop, 1958), but no studies of its effect on cell division in intact roots were listed by Stowe and Yamaki (1957) in their review of the literature on the gibberellins.

In the present study, GA-treated roots showed initial depression of mitosis followed by an after-effect of stimulation of mitosis. The pattern was similar to but not so pronounced as that seen after treatment with IAA. Response to treatment with 1 ppm GA + IAA is similar to that seen with either alone, but their effects are not additive.

At 10 ppm, GA was obviously inhibitory. After 3 days the roots appeared to be dead.

Effects of Indoleacetic Acid

After treatment with 1 ppm IAA the number of mitotic figures present was markedly decreased below the control level for the first 2 days (Table 1). This contrasts with the findings of Burstrom (1942) and Levan (1939) as cited by Aberg (1957) that "the number of mitoses in roots treated with auxin concentrations strongly inhibiting to their longitudinal growth is not decreased during a period of several days." In the present study, 1 ppm IAA was found to inhibit the longitudinal growth of the roots for about 3 days, during which time the characteristic bulbous swelling or "c-tumor" was formed just proximal to the root tip.

After about 3 days, the root resumed elongation, and the tip began to grow away from the tumor. This gross response has previously been noted by a number of workers, and it has been noted that after the initial inhibition there may be subsequent stimulation of gross elongation (e.g. Thimann, 1937). It was found in the present study that mitotic activity exactly paralleled this characteristic total elongation response of the root. There was initial inhibition of mitosis during the period of inhibition of total elongation of the root, followed by a return to the control level at about 3 days. At 5 days, during the time at which stimulation of gross elongation is seen, the number of mitotic figures exceeded that of the control.

At 10 ppm, IAA noticeably inhibited mitosis, and there was no

later release from the inhibition. The roots appeared dead after 3 days.

Interactions of IAA with each of the other substances used here have been reported. In the present study, the initial inhibitory effect of IAA on mitosis was not reversed by its combination with any of the other substances. Only in the combination with GA is the after-effect of stimulation evident.

Effects of Maleic Hydrazide

In some test reactions MH appears to reverse the effects of IAA; in others, to reinforce them. In the present study, it neither reinforced nor reversed the effect of any of the substances used, all of which can promote growth under some circumstances. However, it may have prevented the after-effect of stimulation of mitosis, for this was never seen when MH was present in the combination.

After 48 hours of treatment with MH there appears to be a characteristic release of inhibition of mitosis. This was consistently noted in the data obtained in the present study, and is shown in the data of Greulach and Atchison (1950).

Histological Effects

The bulbous swellings or c-tumors, which form just proximal to the tip of roots inhibited in their longitudinal growth by certain treatments, are the result of a radial expansion of cortical cells proximal to the meristematic region of the root tip. This has been noted previously; in the present investigation it was further noted that the anticlinal walls of these cortical cells appear to be weakened and are often partly disorganized. It is the anticlinal walls that have been stimulated to an increase in area.

In the shoot, the characteristic effect of auxin is the production of cell elongation, in which it is the longitudinal or periclinal wall of the cell which increases in area. In the root, it is the transverse or anticlinal wall which is stimulated to an increase by auxin. This suggests that there may be a difference in composition and/or structure between the periclinal and the anticlinal walls of cortical cells, and that the type of structure which composes the periclinal wall in shoot cells may be the same as, or similar to, the type which forms the anticlinal wall in root cells.

Cells of the rootcap and those of the cortex begin to differentiate very close to the apical initial area. Root tips treated with higher concentrations of IAA showed deleterious effects on these two tissues before the less differentiated tissues were adversely affected. In sections in which the cortex and rootcap cells had lost their capacity to stain and had pycnotic nuclei or no nuclei, the apical initial area, the provascular area, and the dermatogen retained a normal appearance.

It is commonly stated or implied in the literature that the youngest tissues are the most sensitive to auxin. In these sections it appeared that the youngest tissues were more resistant to the deleterious effects of high auxin concentration than were those which had begun differentiation.

In sections of root tips treated with 10 ppm kinetin, almost exactly the reverse condition was seen. Cells in a sharply circumscribed area corresponding to the location of the apical initials had a markedly abnormal appearance, whereas the immediately adjacent cells of the cortex, dermatogen, and nearly all of the provascular area retained a normal appearance. Only the column of xylem mother cells appeared abnormal.

Roots treated with 10 ppm MH showed areas of necrosis, especially in the region of the differentiating vascular cylinder. No especially harmful effects in the meristematic area were noted, in contrast to the findings of Moore (1957) and Gifford (1956).

Clowes (1956) has suggested that there is a "quiescent center" in the area of the apical initials, in which cells normally rarely or never divide. No detailed data on this were gathered in the present study, but it was noted that mitotic figures were very rare in the central part of the apical initial area.

Cytological Effects

Most of the dividing cells had 16 chromosomes at anaphase, but more than this number could be counted in many. An occasional lagging chromosome or bridge could be found in the controls.

No obvious increase in mitotic aberrations was noted after treatment with any of the substances used here with the exception of MH. Roots treated with 10 ppm MH showed mitotic aberrations in about one-third of the dividing cells. Anaphase lagging of chromosomes, bridging, fragmentation, and changes which gave the chromosomes a beaded appearance were seen.

McLeish (1953) found no chromosome breaks after MH treatment of onion root tips, and Greulach and Atchison (1950) found no such aberrations in MH-treated onion roots. Leopold and Klein (1952) and Gifford (1956) reported the presence of mitotic aberrations in MH-treated plant material. The beaded appearance of the chromosomes which was seen in the present study after treatment with MH is shown in Figure 1 in chromosomes which are lagging at anaphase, and Figure 2 shows an anaphase bridge.

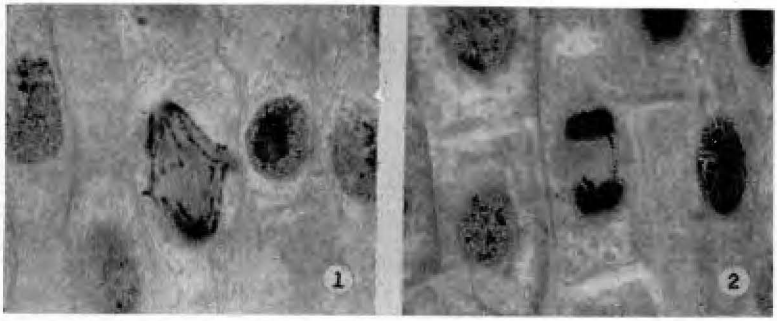

Figure 1. Aberrant mitotic figure seen after treatment with maleic hydrazide. Chromosomes are lagging at anaphase and have a beaded appearance.

Figure 2. Anaphase bridge seen in onion root tip treated with maleic hydrazide. The bridge shows the beaded appearance.

Literature Cited

Aberg, B. 1957. Auxin relations in roots. Ann. Rev. Pl. Physiol. 8: 153-180.

Bradley, M. V. & Crane, J. C. 1957. Gibberellin-stimulated cambial activity in stems of apricot spur shoots. Science 126: 972-973.

Clowes, F. A. L. 1956. Localization of nucleic acid synthesis in root meristems. Jour. Exp. Bot. 7: 307-312.

Gifford, E. M., Jr. 1956. Some anatomical and cytological responses of barley to maleic hydrazide. Amer. Jour. Bot. 43: 72-80.

Greulach, V. A. and Atchison, E. 1950. Inhibition of growth and cell division in onion roots by maleic hydrazide. Bull. Torrey Club 77: 262-267.

——— and Haesloop, J. C. 1958. The influence of gibberellic acid on cell division and cell elongation in *Phaseolus vulgaris*. Amer. Jour. Bot. 45: 566-570.

Guttman, R. 1956. Effects of kinetin on cell division, with special reference to initiation and duration of mitosis. Chromosoma 8: 341-350.

Jensen, W. A. and Pollock, E. G. 1958. Effect of kinetin on the protein and nucleic acid content of root tip cells. Pl. Physiol. (Sup.) 33: xv.

Kaufmann, B. P. and Das, N. K. 1955. The role of ribonucleoproteins in the production of mitotic abnormalities. Chromosoma 7: 19-38.

Leopold, A. C. and Klein, W. H. 1952. Maleic hydrazide as an anti-auxin. Physiol. Plantarium 5: 91-99.

McLeish, J. 1953. The action of maleic hydrazide in *Vicia*. Heredity (Sup.) 6: 125-147.

Moore, R. H. 1950. Several effects of maleic hydrazide on plants. Science 112: 52-53.

Sachs, R. M. and Lang, A. 1957. Effects of gibberellin on cell division in *Hyoscyamus*. Science 125: 1144-1145.

Stowe, B. B. and Yamkai, T. 1957. The history and physiological action of the gibberellins. Ann. Rev. Pl. Physiol. 8: 181-216.

Thimann, K. V. 1937. On the nature of the inhibitions caused by auxins. Amer. Jour. Bot. 24: 407-412.

Torrey, J. G. 1956. Physiology of root elongation. Ann. Rev. Pl. Physiol. 7: 237-266.

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