References and Notes

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2. EPN is the trade name for *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate.
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27 July 1961

Flower Initiation in Excised Stem Disks of Wedgwood Iris

Abstract. The presence of substances promoting flower induction was demonstrated in both scales and buds of Wedgwood iris. The flower-inducing extracts of buds appear to contain gibberellin-like compounds.

In the study of flower-initiation phenomena, more and more use is being made of the technique of aseptic culturing both of whole plantlets (*1*) and of isolated buds (*2*). However, until now only the physiology of flowering of dicotyledons, and in particular of those sensitive to the length of the day, has been studied in this way. For a variety of reasons a day-neutral, monocotyledonous plant—the iris cultivar Wedgwood—was chosen as the test material in the study reported here.

The bulbs of Wedgwood iris are lifted at the end of August. They arrive at the laboratory 4 to 6 days after lifting and are immediately stored at 25.5°C. At this temperature they remain vegetative for more than 10 months. In the bulbs, flowers are initiated during dry storage at 13°C. The transition of the shoot apex from the vegetative to the reproductive stage occurs about 4½ weeks after the beginning of the low-temperature treatment. Shorter exposure to temperature of 13°C and also prolonged storage at 25.5°C result in a threshold condition in which the bulbs are slightly activated. The state of activation is shown by the rate of initiation and development of the flower primordium in the excised shoot apex growing on an agar medium.

The shoot apex was in the vegetative state at the time of excision. The threshold condition was the starting point of all our experiments. The threshold was the result of a combination of a period of storage at 25.5°C and a so-called "pretreatment" at 13°C for various lengths of time. The bulb of Wedgwood iris consists of a short axis, called a stem disk, which carries typical scale leaves, young foliage leaves, and, in the center, the shoot apex. After removal of the scales, the remaining part of the bulb was sterilized for 30 minutes with hypochlorite and rinsed in sterile water. Then, part of the stem disk with the two or three youngest leaf primordia and the shoot apex was excised under aseptic conditions and placed on a nutrient medium. The medium contained macronutrients according to the formula of Knop, micronutrients according to the formula of Heller (*3*), sucrose, and agar. Culturing was done in tubes or petri dishes at 13°C in the dark. The average duration of the experiments was 7 weeks. At the end of an experiment the stage of development of the shoot apex was determined under a dissecting microscope.

Isolated stem disks carrying only the shoot apex and the two or three youngest leaf primordia, and excised from bulbs that had been stored at 25.5°C without any prior treatment at 13°, did not become reproductive when kept on the medium at 13° for a period of 7 weeks. On the other hand, explants carrying either all the young foliage leaves present at the time of excision or a piece of scale, when exposed to temperature of 13° for a similar period of time develop flower primordia. It therefore appears that the presence of young leaves and scales promotes flower initiation. Since the medium contained sugar, it does not seem likely that the effect of leaves and scales is merely that of supplying simple organic nutrients.

The promotive influence of scales on flower formation could also be demonstrated in the absence of any connection of living tissue with the shoot apex. This was done in two ways.

1) An excised stem disk and a piece of scale were placed together on the nutrient medium, with their cut surfaces on the agar. The piece of scale used in these experiments, as in those mentioned above in which the scale was attached to the shoot apex, corresponded to one-fourth of the total mass of the scale material and weighed about 4 g. The weight of the stem disk was about 500 mg. The results are shown in Table 1, as experiments 1 through 4. Although there was no union of tissue between the epidermis of the scale and the cut surface of the stem disk, the presence of the scale fragment induced flower initiation in 60 to 100 percent of the explants.

In comparing the results of these experiments, it should be borne in mind that they were carried out at various times—that is, after various periods of storage at 25.5°C. Experiment 2 was carried out in January, experiment 1 in April. So the bulbs of experiment 1 had been exposed to a temperature of 25.5°C almost twice as long as those of experiment 2 and were more acti-

Table 1. Influence of scales and bud extracts on flower induction in excised stem disks of Wedgwood iris. Treatment A: bulbs exposed at 13°C prior to, respectively, incubation of scales and extraction of flower-inducing substances. Treatment B: bulbs exposed at 13°C prior to excision of test stem disks. N_t, total number of stem disks; N_r, number of stem disks that became reproductive. In experiments 1 through 4, scales and apexes were incubated at the same time; in experiments 5 and 6, scales were incubated for 1 week prior to the incubation of apexes.

Experiment	Experimental group				Control group	
	Treatment at 13° (wk)		N_t	N_r	N_t	N_r
	A	B				
			Influence of scales			
1			10	6	12	0*
2			9	2	13	0
3	1		11	11	19	13
4	2		23	14	15	2*
5	1		13	5	32	0*
6	3		13	3	32	0*
			Influence of bud extract			
7	3	1	24	7	30	2†
8	4	1 4/7	23	10	30	2*

*$P < .01$. †$P < .05$.

vated when stem disks and scales were excised. Experiment 4 was performed in October, experiment 3 in January; this explains the differential effect of the 13°C treatment. It appears that the length of the 13° treatment required to obtain a threshold condition in the bulbs is strongly dependent upon the length of the preceding storage period at 25.5°C.

2) Excised stem disks were placed on a medium on which a scale fragment had previously been incubated. As shown in Table 1, experiments 5 and 6, some of these stem disks became reproductive, while the controls did not.

On the basis of these results attempts were made to extract and identify the active material. It was assumed that this material would be more highly concentrated in the apexes and the primordial leaves than in the scales. Cold methanolic extracts of the buds of bulbs that had been stored at 13°C for varying periods of time were prepared by the method of Harada and Nitsch (*4*). *Bud* in this connection signifies that part of the bulb that remains after removal of the scales. The extract was evaporated to dryness at 40°C under reduced pressure. The residue was dissolved in water, sterilized by filtration, and incorporated into the nutrient medium. The first tests were made with extracts of buds of bulbs that had been stored at 13°C for 3 and 4 weeks. After such storage the shoot apexes of these bulbs are still vegetative in appearance, although physiologically the transition to the reproductive state may already be in progress. This time the explants were more severely trimmed, weighing only about 30 mg. They were cultured in small tubes containing 5 ml of nutrient agar. The ratio of the number of extracted buds to the number of explants was 1 to 1. As shown in Table 1 (experiments 7 and 8), after 7 weeks a greater percentage of apexes had become reproductive on the medium containing bud extract than on the control medium.

The next step was to chromatograph the crude methanolic extracts in 80-percent isopropanol on paper strips, according to the method of Harada and Nitsch (*4*). The Avena first-leaf-section test was applied to detect eventual gibberellin-like substances. On the strength of these determinations it appears that the buds of iris bulbs contain several gibberellin-like substances. During the first 4 weeks of the 13°C treatment the main growth substances are those having R_F values of 0.2 to 0.4 and 0.6 to 0.8, respectively, in 80-percent isopropanol. Gibberellic acid (*5*), which has an R_F of 0.45, did not seem to be present in the extracts, although in earlier experiments (*6*) this growth substance had been shown to exert a strong flower-inducing effect.

In extracts of buds of bulbs that had been stored at 13°C for 7 weeks and thus were definitely reproductive, the fraction with R_F of 0.2 to 0.4 was extremely active in the Avena first-leaf-section test, whereas no activity was found between R_F 0.6 and 0.8. In preliminary experiments the capacity of these fractions to induce flower development was tested. The extract was chromatographed, and the regions of the strips corresponding to R_F values 0.2 to 0.4 and 0.6 to 0.8, respectively, were eluted. The eluates were sterilized by filtration and incorporated into the nutrient agar. On these media small explants were incubated, as in experiments 7 and 8. Each tube, carrying one explant, contained an amount of extract originating from one bud. The results seem to indicate that flower initiation is promoted by the fraction having an R_F value of 0.2 to 0.4 in 80-percent isopropanol.

These findings suggest that in the bulbs of Wedgwood iris, flower induction is associated with changes in endogenous gibberellin-like substances. By systematically screening extracts prepared at several different times in the course of the flower-inducing 13°C treatment, both by means of a test specific for gibberellins and with the technique of aseptic culturing of apexes, it should be possible to draw more specific conclusions about the mode of action of the gibberellin-like substances in floral initiation.

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3 August 1961

Leukemogenesis by Urethan in C57Bl Mice Bearing Isologous Tissues from X-Irradiated Mice

Abstract. A single exposure of mice of a low-leukemic strain (C57Bl/6) to 400 r of total-body x-irradiation caused the early appearance of a factor which, though not itself capable of inducing leukemia in adult C57Bl recipients, did cause its appearance when the recipients also received urethan by injection. Positive results were obtained with seven different tissues from irradiated animals, whether urethan was injected 1 day or 30 days after the irradiation. No such effect was obtained in either series in the absence of urethan treatment. When urethan was injected into normal C57Bl mice, or into mice previously implanted with tissues from nonirradiated mice, only marginal leukemogenic activity was evinced. The possible involvement of a precursor virus is discussed.

Experimental leukemogensis by x-irradiation differs from other forms of carcinogenesis with respect to the involvement of several complicating factors. (i) Radiation leukemogenesis does not operate in thymectomized mice but becomes again effective when normal thymus is reimplanted after the irradiation (*1*). Thus, the eventual target organ (the thymus) is required for leukemogenesis, though it need not be acted upon by the inciting agent (x-irradiation). (ii) Radiation leukemogenesis is interfered with by the presence of normal bone marrow, as evidenced by the ineffectiveness of irradiation when one limb is shielded (*2*), and by the marked depression of leukemogenesis by total-body irradiation when normal, isologous bone marrow is injected subsequent to the irradiation (*3*). (iii) While the presence of a leukemia virus, demonstrable in mice of a high-leukemic strain (*4*), is not demonstrable in untreated mice of a low-leukemic strain (see *5*), it becomes demonstrable in mice of a low-leukemic strain after irradiation, either after (*6*) or shortly before (*7*) the disease appears.

Radiation leukemogenesis can be augmented by urethan treatment when this is given either simultaneously with the irradiation (*8, 9*) or 2 weeks later, but not when it is given 2 weeks prior to the irradiation (*9*). The possibility that the urethan effect might be due to a further depression of the bone marrow has been excluded (*10*). It would seem (*9, 10*) that urethan operates as a "promoting" factor in leukemogensis, with x-irradiation (in the low-dose ranges used) acting as "initiating" factor, in