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Studies on the role of kinetin and vitamin E in the flowering of the cold requiring plant (*Cichorium intybus L.*) and the long day plant (*Arabidopsis thaliana L.*) grown in non-inductive conditions

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There is much evidence for the important role of gibberellin played in the flowering processes of plants. This substance may induce the flowering in some cold-requiring and long-day plants grown under non-inductive conditions (for review — Lang and Reinhard 1961).

The flowering of plants may be also induced by kinetin and vitamin E.

The stimulating influence of kinetin on the flowering of short-day plants was showed by Lona and Bocchi (1957, 1959) in experiments with *Perilla*, and also by Ogawa (1961) in experiments with *Pharbitis nil*. Chailakhian and Butenko (1959) reported also the fact that *Perilla*, cultivated *in vitro* may produce flower buds as an effect of kinetin and adenin treatment, even on long day conditions.

A certain role in the process of flowering play also tocopherols (vitamin E). This was shown by Sironval (1954, 1957, 1960) in experiments with *Fragaria vesca*. He maintains that plants growing on long-day produce more vitamin E. The treatment of plants with vitamin E leads to the initiation of flower primordia. This vitamin stimulates also the flowering of *Fragaria* on short day when the process of flowering has been already initiated. According to the opinion of this author, vitamin E plays an essential part in the process leading to flower formation in plants requiring photoperiodic induction (Sironval 1960). According to Bouillenne (1961) some complex compound containing vitamins E and K as well as sterides is responsible for the generative development of plants. In the opinion of Sironval, the above named substances are formed in short-day plants on short day and in long-day plants on long day. Bruinsma and Patil (1963) have also underlined the importance of vitamin E in the process of flowering. Unvernalized Petkus winter rye treated with tocopherol at the phase of 6—10 leaves, turned from the vegetative into the generative stage.

The results of our experiments with *Cichorium intybus* (1964), i.e. cold-requiring plant, and with *Arabidopsis thaliana* (1965) a long-day plant have shown that kinetin and tocopherol can induce flower formation of these plants grown under non-inductive conditions similar as gibberellin does.

We have concluded that gibberellin cannot be regarded as a specific substance having the ability to „substitute" cold in cold-requiring plants or long day in long-

-day plants. We also pointed out that it is possible that the role of kinetin and vitamin E may control the level of endogenous gibberellin in plants.

The aim of this work was to explain this problem.

METHODS

The cold requiring *Cichorium intybus* L. and the spring *Arabidopsis thaliana* Heynh. a long-day plant, have been used for these experiments. The plants have grown in non-inductive conditions at a temperature of 26—27°C day, and 20—21°C night on long day (16 hrs). Light sources were „day light" fluorescent tubes of an intensity of 4000 lux. The plants have been cultivated in boxes with garden soil. They were planted on the 22.12.1965.

Kinetin and vitamin E was applied as described in the previous papers (Michniewicz, Kamieńska 1964, 1965). The treatment with the active substances started at the time when the plants were in the rosette phase of 6—8 leaves. Kinetin was applied to the tips of the plants every time at the amount of 1 μg, and tocopherol at 10 μg per plant per application. *Cichorium* was treated 15 times and *Arabidopsis* 10 times every other day. All chemicals were produced by Merk's Company.

Whole plants in the vegetative phase treated 5, 10 or 15 times in the case of *Cichorium*, and 5 or 10 times in the case of *Arabidopsis* were taken for analysis two days after the final treatment.

Frozen plant material (30 g of fresh weight of *Cichorium* or 6 g of *Arabidopsis*) was extracted in 70% acetone for 48 hrs in room temperature. The acetone was evaporated and the water residue was acidified to pH 2 and extracted with ethyl acetate. Fractions of ethyl-acetate were afterwards extracted with phosphate buffer of pH 7 and acidified to pH 2, and then extracted with ethyl acetate. The ethyl acetate was evaporated to dryness and the residue was diluted in 1 ml of acetone and chromatographed. All evaporations were made in a temperature of 38—40°C.

Paper and thin layer chromatography were used. The equivalent of 3 g of fresh weight was put on the chromatograms.

The methods of paper chromatography were described in detail in a previous report (Michniewicz and Kopcewicz 1966). As solvent system were used n-butanol, acetic acid and water (19 : 1 : 6 v/v). For thin-layer chromatography were used glass plates 17×17 cm. They were covered with a coat of silica-gel (Merk's production) according to Stahl (1962). Layers of gel were activated in a temperature of 105°C for 30 min. Chromatograms were developed up to a length of 12 cm. As a solvent system were used : benzene, acetic acid (10 : 3 v/v) (Sembdner et al. 1962).

The content of gibberellin-like substances was estimated by the test of first oat leaf (Michniewicz 1961) in the case of the paper chromatography method and also by the lettuce hypocotyle test (Frankland and Wareing 1960), when the thin-layer method was used.

All those experiments were repeated four times. Significant differences were defined by estimating L.S.D. at $P = 0.01$.

RESULTS AND DISCUSSION

As already reported, the treatment of *Cichorium intybus* and *Arabidopsis thaliana* with kinetin and vitamin E affected the flowering of these plants in non-inductive conditions (Michniewicz and Kamieńska 1964, 1965). *Cichorium*, which grew on long day at a temperature making a flowering normally impossible, produced flowering at 40—60% after being 15 times treated with 0.1—0.001 μg of kinetin or 10—30 μg of α-tocopherol-acetate.

Plants which were treated with kinetin and vitamin E were taken for analysis during the vegetative phase before bolting. All plants, the control as well as the experimental ones, were in the rosette phase and did not differ from each other with regard to the number nor to the size of leaves or the content of dry weight.

Data concerning the influence of kinetin and vitamin E on the level of endogenous gibberellin are given in table 1. The table contains only the results obtained by paper chromatography. Using the method of thin-layer chromatography the results were almost identical.

Judging on the results summarized in table 1 it is clearly seen that the treatment of *Cichorium* with kinetin, as well as α-tocopherol acetate, effected a decrease of the level of gibberellin-like substances in all experiments.

In the case of *Arabidopsis*, the decrease in the level of gibberellin was obtained in those plants which were treated five times with kinetin. In the later phase, when the plants were treated 10 times with this growth substance, the level of gibberellin showed a small but steady increase in relation to the control plants. Unfortunetaly, the influence of vitamin E on the level of gibberellin in *Arabidopsis* could not be examined, as not enough material for a correct analysis was then available.

The results obtained now do not confirm the previously made supposition (Michniewicz and Kamieńska 1964, 1965) that the influence of kinetin and vitamin E in the process leading to the flowering of *Cichorium* and *Arabidopsis* might be indirect and may rely on the increase of the level of endogenous gibberellins. The results obtained in the present paper point rather towards the assumption that these processes may occur independently on the level of gibberellin in a plant. This is supported by the fact that the amount of gibberellin clearly decreased under the influence of kinetin and vitamin E in *Cichorium*, but increased in *Arabidopsis* treated repeatedly ten times with kinetin.

As underlined before, the plants taken for analysis, control as well as experimental, were in the vegetative phase. May be that a comparison between plants in the vegetative phase and those in the generative one would give some other results (cf. Lang and Reinhard 1961).

A comparison of plants being at different developing phases would however not supply the answer to the question whether gibberellins are the cause or the results

Table 1

Content of gibberelin-like substances expressed as equivalent of GA_3 activity in 100 g fresh weight (I) and dry weight (II) in plants treated with kinetin and vitamin E.

Species	Date	Control		Kinetin			Vitamin E		
		I	II	Amount of applied substance in μg	I	II	Amount of applied substance in μg	I	II
Cichorium intybus	Feb. 19	0.2514	1.4165	0	0.2514	1.4165	0	0.2514	1.4165
	March 1	0.5861	2.8412	5	0.0045	0.0228	50	0.0020	0.0103
	March 11	0.2985	1.6475	10	0.1930	1.4010	100	0.0722	0.5096
	March 21	0.0343	0.3062	15	0.0022	0.0210	150	0.0055	0.0367
Arabidopsis thaliana	Feb. 19	0.4070	4.1111	0	0.4070	4.1111	—	—	—
	March 1	0.4616	4.7108	5	0.0148	0.1542	—	—	—
	March 11	0.0101	0.1124	10	0.0829	0.9242	—	—	—

L. S. D. at P = 0.001 (fresh weight)
Cichorium — 0.0295
Arabidopsis — 0.0243

of the turning of plants from the vegetative into the generative stage of development.

The facts here presented suggest that the process leading to the flowering of plants in non-inductive conditions may occur by an increased amount of endogenous gibberellin introduced egzogenously and also at a lowered amount of this growth substance. It may be then supposed that flowering can be brought about not only by the action of a gibberellin, but it may be also induced in various ways, independent on gibberellin.

SUMMARY

Cichorium intybus, a cold-requiring plant, and *Arabidopsis thaliana*, a long-day plant, are able to flower in non-inductive conditions not enly under the influence of gibberellin but also as a result of treating them with kinetin and vitamin E.

The treatment of *Cichorium* and *Arabidopsis* with kinetin or vitamin E, in non-inductive conditions, caused in the vegetative plants, a decrease of the level of endogenous gibberellin in *Cichorium*, and an increase of this growth substance in *Arabidopsis*. The plant may flower at an increased as well as at a decreased level of gibberellin.

It is supposed that the turning of a plant from the vegetative into the generative stage of development in *Cichorium* and *Arabidopsis* can be brought not only by the action of a gibberellin, but it may be also induced in various ways, independent on gibberellin.

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Badania nad rolą kinetyny i witaminy E w zakwitaniu Cichorium intybus — rośliny wymagającej termoindukcji i Arabidopsis thaliana — rośliny dnia długiego, rosnących w warunkach nieindukujących

Streszczenie

Cichorium intybus — roślina wymagająca do zakwitania termoindukcji — oraz *Arabidopsis thaliana* — roślina dnia długiego — zakwitają w warunkach nieindukujących nie tylko pod wpływem giberelliny, lecz mogą zakwitnąć w tych warunkach również na skutek traktowania ich kinetyną i witaminą E.

Traktowanie *Cichorium* i *Arabidopsis* w warunkach nieindukujących kinetyną lub witaminą E, prowadzące w rezultacie do zakwitania, wywoływało u roślin, będących jeszcze w fazie wegetatywnej, obniżenie poziomu endogennej giberelliny u *Cichorium* i zwiększenie ilości tej substancji wzrostowej u *Arabidopsis*.

Zakwitanie zachodzić może zarówno przy zwiększonej jak i przy zmniejszonej ilości giberelliny.

Wysunięto przypuszczenie, że przejście roślin z fazy wegetatywnej do generatywnej nie jest uwarunkowane u *Cichorium* i *Arabidopsis* działaniem giberelliny i może zachodzić innymi drogami.