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Chenopodium rubrum as a Model Plant for Testing the Flowering Effects of PGRs

J. ULLMANN, FRIDETA SEIDLOVÁ, J. KREKULE and LIBUŠE PAVLOVÁ

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Ke dvoru 15, 166 30 Praha 6, Czechoslovakia

Abstract. In short-day plant *Chenopodium rubrum* the photoperiodic requirement for flowering increases from one short day at the age of four days to two or three short days at the age of five to seven days. The photoperiodic requirement decreases again to one short day between the 10th and 12th day of cultivation. This feature, together with endogenous circadian rhythmicity of flowering, enabled us to test the effects of PGRs under different morphogenetic patterns. Representative PGR effects on flowering are quoted.

Chenopodium rubrum is a qualitative SDP which can be induced to flowering already at cotyledonary stage (CUMMING 1959). It has been found that one short day brings about flowering in three to four days old plants (SEIDLOVÁ and KREKULE 1973), whereas six to eight days old plants require two to three short days (SEIDLOVÁ 1980). Transition to flowering thus proceeds under different pattern of apex growth. This, together with changes in responsiveness due to phases of endogenous rhythmicity and the number of inductive cycles, provides a useful diversity of situations for testing the stimulatory and inhibitory effects of PGRs on flowering.

The effects of phytohormones and other PGRs on floral induction and differentiation were investigated in *Chenopodium rubrum* by *e.g.* SEIDLOVÁ (1980), KREKULE *et al.* (1985) and ULLMANN *et al.* (1985). The effects of florigenic extracts from flowering tobacco plants have been also tested using *Chenopodium rubrum* (CHAÏLAKHYAN *et al.* 1977). The aim of the present paper is to characterize the developmental pattern of *Chenopodium* as related to plant age and endogenous rhythmicity of flowering and to illustrate the resulting differences in response to PGR treatment.

MATERIAL AND METHODS

A qualitative short-day (selection 374) *Chenopodium rubrum* (CUMMING 1959) was used in our experiments. Uniform germination was promoted by temperature fluctuations (Fig. 1). Selected plantlets were either cultivated as hydroponics or in perlite. Half strength Knop's solution and small plastic

vessels were used in both cases. The seedlings were cultivated in small-volume growth chambers at a temperature of 20 ± 1 °C and continuous illumination of *ca.* 8000 lx provided by fluorescent tubes. Photoperiodic treatment is described in Results. The substances were applied as a droplet (3 μl) of water solution to the plumule or to the cotyledon, and *via* roots in plants cultivated as hydroponics. The growth response and the flower differentiation of the shoot apical meristem were evaluated directly under a stereo-microscope or using drawings from projection-microscope or from photographs.

RESULTS AND DISCUSSION

As seen in Fig. 1 the first maximum of photoperiodic response occurs at the age of four days, immediately after the testa is shed. Two days later two short days are required to induce flowering. Such a rise in photoperiodic requirement with age is rather exceptional and was observed *e.g.* in *Phar-*

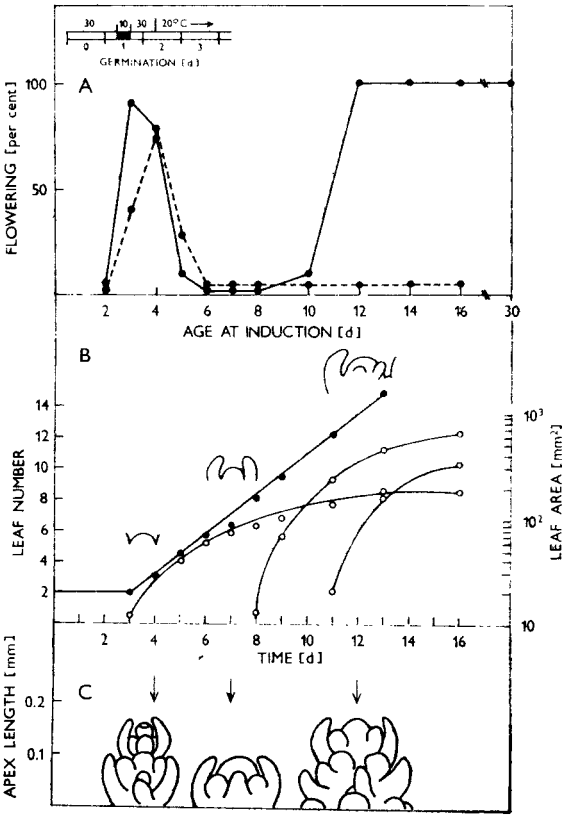

Fig. 1. Age-dependence of flowering response in plants induced by one short day ($\overline{12}$ h + 12 h). — A. Flowering of plants grown in perlite (solid line) or as hydroponics (broken line). — B. Growth pattern prior to induction (plants cultivated under continuous light); apex shape and the rate of leaf initiation (closed circle); growth rate of cotyledons, and of the first two leaf pairs (open circle). — C. Apical meristem five days after one inductive short day ($\overline{12}$ h + 12 h) given at the age of four, seven or twelve days, respectively.

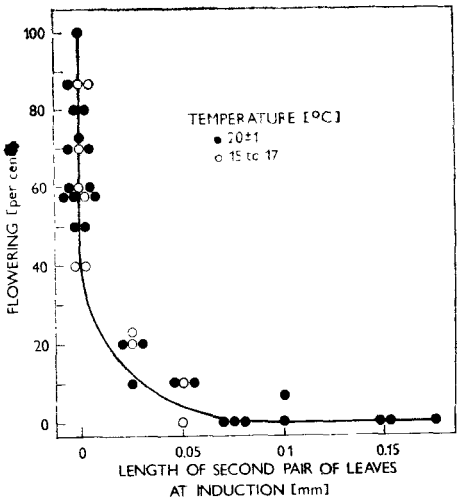

Fig. 2. Flowering response to one inductive short day ($\overline{12h}+12h$) as related to leaf formation before induction. Closed circles refer to results of experiments performed at temperature 20 ± 1 °C; open circles refer to experiments at 15—17 °C. (A delay of one to three days in leaf formation resulted from the lowered temperature.)

bitis (KUJIRAI and IMAMURA 1958). It coincides in *Chenopodium* with the period of highest growth rate of cotyledons (Fig. 1B) and with the differentiation of the second leaf pair, whereas the first leaf pair is already present in the seed. The correlation between plastochron duration and the number of inductive cycles needed for induction of flowering (JACOBS 1972) was not confirmed for *Chenopodium*. The capacity to induce flowering by one short day is restricted in *Chenopodium* to the early phase of germination and declines with the progress of leaf formation, irrespective of chronological age. This may be inferred from the fact that any delay in leaf initiation, *e.g.* due to temperature decrease (Fig. 2) will postpone the rise in photoperiodic requirement.

The decrease of photoperiodic requirement with plant age found in *Chenopodium* approximately at the age of 10 days is of general occurrence (LANG 1965). It is assumed to be linked with attainment of the intermediate stage (BERNIER *et al.* 1981) of apical meristem or with the drop in inhibitor level in leaves and cotyledons (PODOLNY *et al.* 1981). In *Chenopodium* the growth of cotyledons is accomplished at that time and their size is equal to that of the first leaf (Fig. 1B). The restoration of one-cycle requirement also coincides with the replacement of cotyledons by leaves as receptors of the photoperiodic signal. This was shown by experiments with organ removal. Thirteen days old plants deprived of cotyledons and subjected to one short day flower at the same time as intact control plants, whereas removal of the first leaf pair at this age inhibits flowering.

Plants induced at different age differ in morphogenetic pattern (Fig. 1C). A minimum number of leaf and bud primordia is formed whereas the rate of floral transition is rapid in very young plants induced by one short day. Terminal flower can be distinguished five days after induction. At the age of 12—14 days numerous leaves and buds are already formed. A branched inflorescence is initiated in plants induced by one short day at this age and the differentiation of reproductive organs lasts up to 9—10 days in this situation. One short day will bring about only partial induction in six days old plants

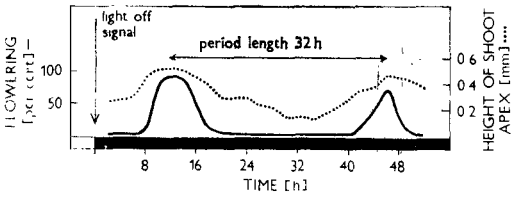

Fig. 3. Endogenous rhythm of flowering in 15 days old plants cultivated in perlite.

and a reversion to vegetative state is observed (SEIDLOVÁ 1980). Three short days are sufficient to induce full flowering in such plants (Fig. 5).

Although the physiological mechanism of age-dependent changes in photoperiodic requirement of *Chenopodium* is far from clear, the phenomenon in itself may be effectively used in investigating flowering. Within one genotype it is possible to compare the flowering response to various treatments under different patterns of morphogenesis.

Endogenous Circadian Rhythmicity of Flowering and Photoperiodic Induction in *Chenopodium*

An oscillatory type of endogenous circadian rhythm of flowering has been described in *Chenopodium rubrum* (CUMMING 1969). It is entrained by the light-off signal in plants induced by one short day. Fig. 3 presents its basic characteristics which do not change with plant age (ULLMANN and KREKULE, in preparation). The optimal length of dark period of the inductive short day is derived from the character of the rhythm and corresponds to the position of the first peak of capacity for flowering, *i.e.* to 12—14 h of darkness. However, this optimal length may change in subsequent inductive cycles, providing that plants are induced to flower by more than one short day. This is the case of plants five to ten days old, being in the period of increased photoperiodic requirement. The location of the peak is then shifted due to the interference of two signals — light off and light on (rephasing of the cycle). Only with a photoperiod exceeding 12 h does the shift not occur. Both situations are illustrated in Fig. 4.

The above data as well as practical considerations led us to suggest optimal inductive regime as $\overline{12}$: 12. As inferred from the character of the rhythm, dark periods shorter or longer than the optimal are less efficient in inducing flowering. This feature may be used to manipulate experimentally the degree of flowering. Knowledge of periods of highest response to red light breaks which cancel flowering (15′—20′ at 4th and 8th hour of darkness)

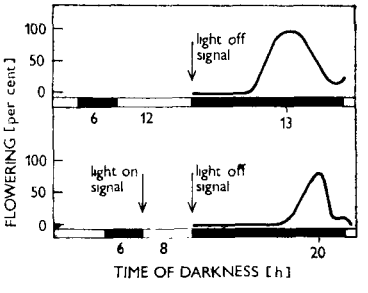

Fig. 4. Changes in the position of the peak of flowering capacity due to different lengths of the preceding photoperiod.

made it possible to introduce control treatment: plants receiving an equal amount of light energy as the induced ones, but remaining in vegetative state.

Some Effects of Phytohormones and Other PGRs on Flowering

The effects of PGR on flowering in *Chenopodium rubrum* were localized both in shoot apices and leaves and cotyledons (KREKULE *et al.* 1985). Some results indicate time-dependence of PGR effects which may be linked to phases of the endogenous rhythm of flowering (*e.g.* KING 1975, ANDREAE 1975). This stresses the need to consider the phase of endogenous rhythmicity in PGR treatments.

The action of PGRs in flowering is strictly stage-dependent. This was demonstrated in *Chenopodium* by SEIDLOVÁ (1980) as auxin treatment resulted either in stimulation or in inhibition of flowering depending on the stage of apical meristem differentiation. For timing the PGR effects the particular stage of apical meristem in the period between the beginning of photoperiodic induction and flowering is of the utmost importance. Its character also depends on the number of inductive cycles and the endogenous rhythmicity of flowering. Surprisingly enough, the chronological age of plants prior to induction is of lesser importance, *i.e.* what matters is the stage achieved, not the age in itself.

Detailed analysis of changes in the growth pattern of apical meristem and the respective primordia due to PGR treatment has demonstrated that at cellular level the effects of a given substance are strikingly similar. This holds true even for plants in the vegetative stage. Thus, auxins mainly inhibit cell division in axillary meristems, cytokinins enhance cell division, GA_3 and ABA change the direction of cell growth with predominance of the longitudinal in the former and the transversal in the latter case (SEIDLOVÁ 1980). Such rather non-specific growth effects result in stimulation or inhibition of flowering depending on their integration within the particular stage of floral transition.

None of the PGR used in *Chenopodium* was able to substitute for at least partial photoperiodic induction of flowering. This was one of the reasons to using *Chenopodium* as a model for testing the florigenic activity of extracts from SD and LD tobacco (CHAÏLAKHYAN 1977). The application of such extracts in older plants of *Chenopodium* requiring one short day brought about flowering under strictly non-inductive conditions, whereas in plants with a higher photoperiodic requirement (three SD) partial substitution of inductive conditions was observed.

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Fig. 5 is at the end of the issue.

BOOK REVIEW

JAMESON, C. W., WALTERS, D. B.: CHEMISTRY FOR TOXICITY TESTING, — Butterworth Publishers, Boston — London — Sidney — Wellington — Durban — Toronto 1984. 231 pp. Hardcover £ 32.50.

It is estimated that several thousand different chemicals are currently being used in various industrial and agricultural operations, and that hundreds of new chemicals are being introduced into use each year. In the past years, significant advances have been made in the development of methods to assess toxic hazard of these man-made and naturally occurring chemicals. This 18-chapter book, presenting state-of-the-art reports on analytical chemistry requirements for many aspects of *in vivo* toxicity studies, is arranged into four parts. The introductory part covers general chemistry considerations concerning toxicity studies. Included are chapters on analytical chemistry requirements for toxicity testing, structure-activity prediction of the carcinogenicity of chemicals and on problems of testing commercial-grade chemicals. Part II deals with the problem of dosage mixing and analysis in rodent feed, methods used in formulation of insoluble and immiscible test agents and in determination of the stability of chemical/vehicle mixtures. Airborne toxic agents, in both gaseous and particulate form, represent a serious hazard to humans. Part III focuses on generating and monitoring test atmospheres, *e.g.* of aerosoles in inhalation chambers, of combustion products, degradation test products *etc.* The last part of the publications discusses the evaluation of chemistry data and management in toxicity testing programs. The chapters in this book are adapted presentations from a symposium: Chemistry and Safety for Toxicity Testing of Environmental Chemicals, Las Vegas, Nevada, 1982.

The publication will meet the needs of all those engaged in testing the toxic hazards of environmental chemicals.

J. VELEMÍNSKÝ (*Praha*)

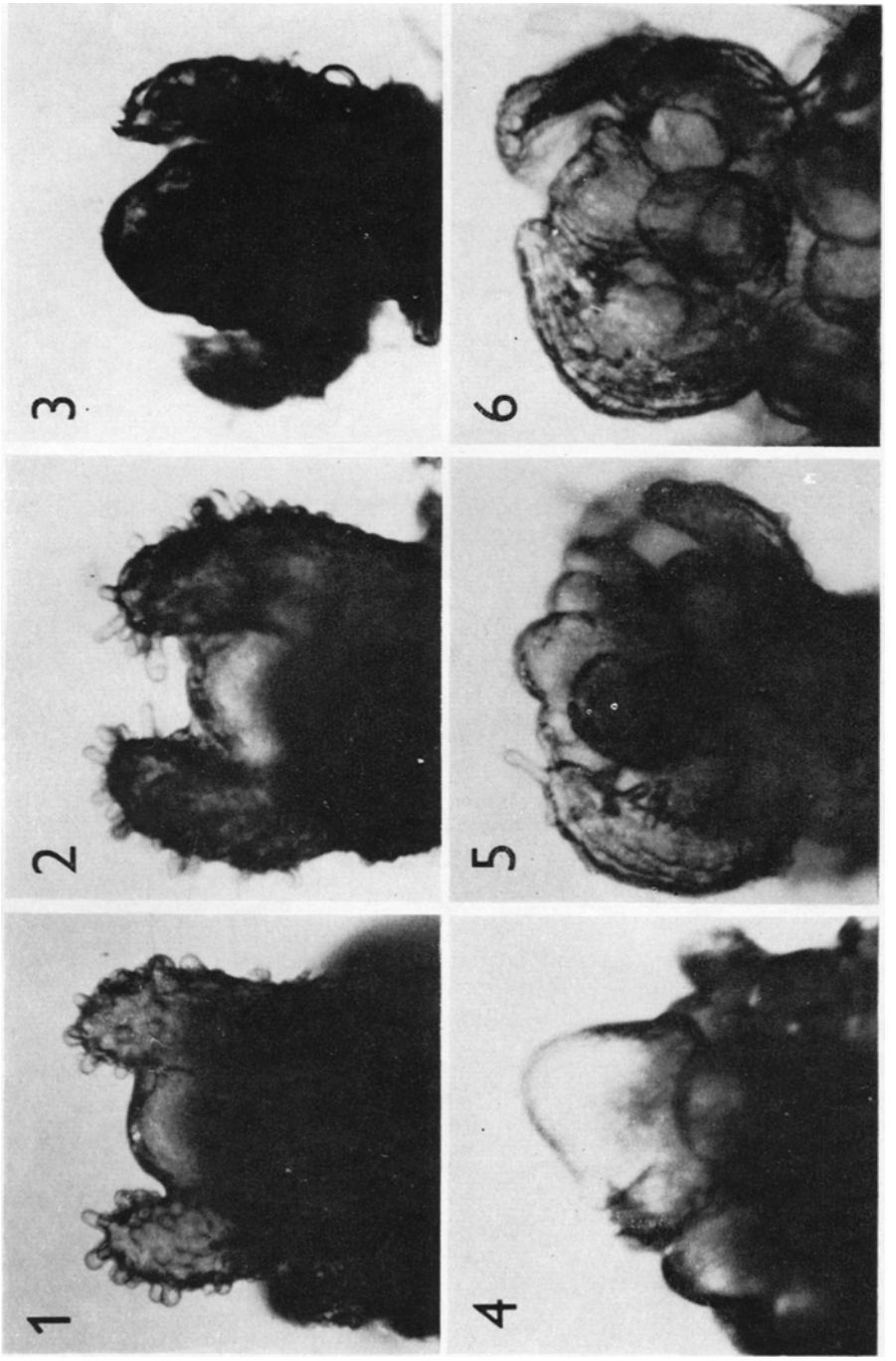

Fig. 5. The development of *Chenopodium rubrum* apex in plants induced by three short days ($\overline{16}$h+8h). — 1,2 — leaf formation; 3,4 — branching; 5 — beginning of flower differentiation; 6 — flower bud.