BIOLOGIA PLANTARUM (PRAHA)

27 (4—5) : 310—317, 1985

Auxin in Flowering of Short-Day and Long-Day *Chenopodium* Species

J. KREKULE, LIBUŠE PAVLOVÁ, DAGMAR SOUČKOVÁ and IVANA MACHÁČKOVÁ

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

Abstract. The fluctuation of free IAA under 16 h dark period in shoots (receptor organs of photoperiodic induction) and roots of the short-day plant (SDP) *Chenopodium rubrum* and in shoots of the long-day plant (LDP) *Chenopodium murale* is very similar. The data reflect the general adjustment of auxin level to day-length rather than changes due to floral induction. However, the shift in phasing of the circadian rhythm of flowering was accompanied by a change in the position of the' troughs' of free IAA levels indicating a possible relationship between the two processes. Periods of higher sensitivity to application of IAA ($3 \cdot 10^{-4}$M) inhibitory to flowering have been observed both during the endogenous rhythm of flowering in the SDP *C. rubrum* and during induction by days of continuous illumination in the LDP *C. murale*. There exist common traits in the response of LDP and of SDP *Chenopodium* to auxin treatment. Aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, counteracted some flowering inhibitory effects of IAA when applied simultaneously with it. This suggests that auxin effects in modifying flowering might in fact be due to ethylene.

Since the first report by DOSTÁL and HOŠEK (1937) on auxin inhibitory action in flowering of *Circaea intermedia*, this phytohormone has been generally recognized as part of the flowering control mechanisms. Its status ranged from that of a specific component of floral stimulus to a flowering modifying agent exhibiting pharmacological effects. In general, auxin was found to inhibit flowering in short-day plants and to be ineffective or to promote flowering in long-day plants (most of the older literature was reviewed by LANG 1961). For several reasons interest in auxin revived in recent years. The introduction of explants as models for studying flowering (*e.g.* AKSENOVA *et al.* 1972) once again confirmed profound effects of applied auxin on flower initiation and morphogenesis. Furthermore, sites of exogenous auxin action in flowering were recognized at least at the anatomical level. It was observed (SEIDLOVÁ and KHATOON 1976) that auxin suppresses the activity of axillary meristems which are released from apical dominance by treatments enhancing or inducing flowering, notably by photoperiodic induction. The role of auxin in changing membrane properties may also be relevant to some of its effects on floral activity, as fluctuations in membrane state has been considered as a basis of most phenomena of endogenous rhythmicity (VANDEN DRIESSCHE 1980), including those of flowering.

In order to study the physiological role of auxin in flowering the following approaches were adopted, based on our previous results. We tested the already established relation between a decreasing level of free IAA in shoots and a high capacity of flowering (PAVLOVÁ and KREKULE 1984).

Moreover, we investigated whether or not the rhythmic fluctuation of endogenous IAA reflects changes in responsiveness to exogenous IAA. Also we wished to know whether there exists any similarity in response to IAA treatment in SD and LD *Chenopodium*. The flowering promoting activity of auxin in *Bromeliaceae* is due to enhanced ethylene synthesis. Thus, we tried to find out whether some other auxin effects, namely the inhibitory ones, are mediated by ethylene.

MATERIAL AND METHODS

Material

Seedlings of the quantitative short-day plant *Chenopodium rubrum* (selection 374) and quantitative long-day plant *Chenopodium murale* (selection 197) were used.

Cultivation and Flower Induction

Both species were cultivated in small volume growth chambers at 20 °C. Illumination was provided by day-light fluorescent tubes (55 Wm^{-2} at plant level). The seedlings were grown in half-strength Knop's solution either as hydroponics or in perlite. One to three short days (the critical photoperiod for flowering is 16h) are needed for complete flowering in *C. rubrum* and its response is strictly age dependent (ULLMANN *et al.* 1985). *C. murale* (LDP) is induced to flower by eight to ten days of continuous illumination. The response to the photoperiod is not age dependent. The seedlings were cultivated before and after induction: under continuous illumination in SDP *C. rubrum* and under eight hours short day in LDP *C. murale*. Unlike the majority of LDP *C. murale* does not form leafy rosettes under vegetative conditions. The age of plants and conditions of induction are given with each particular experiment.

Application of Chemicals and Scoring of Plants

IAA and AVG were applied as a 3 µl droplet of aqueous solutions to cotyledons, leaves or plumules at the concentrations indicated for each experiment. The concentration used was most effective in inhibiting flowering without interfering apparently with the growth rate of plants. Ten plants from each treatment were dissected under a stereo-microscope and scored for percentage of flowering and length of the stem apical meristem, which is positively correlated with the degree of induction. The time of evaluation is given in each experiment.

Estimation of Endogenous Free IAA

Endogenous free IAA was estimated as α-pyrone by the fluorimetric method (MOUSDALE *et al.* 1978). A known activity of ^{14}C-IAA was added to the extracts as the internal standard and the losses due to purification were evaluated (PAVLOVÁ and KREKULE 1984).

Uptake and Transport of ^{14}C-IAA

To quantify the uptake of exogenous IAA and to follow its transport, experiments with ^{14}C-IAA were performed. Labelled IAA was applied in the same way as non-radioactive material and the radioactivity distribution between different organs was evaluated using scintillation counting.

RESULTS

Fluctuation of Endogenous Free IAA under Photoperiodic Conditions

Free IAA fluctuation was estimated under conditions inductive for flowering in SDP *Chenopodium rubrum*, *i.e.* under 16 h darkness and 8 h light. We wished to know whether the pattern of IAA changes might be specific

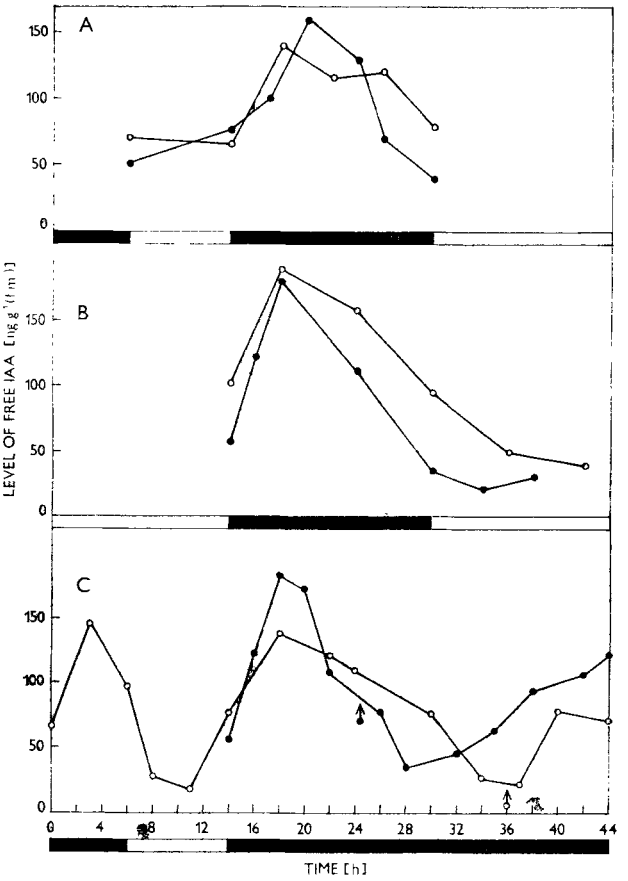

Fig. 1. Level of free IAA in six day old seedlings of *Chenopodium rubrum* and in seven day old seedlings of *Chenopodium murale*. — A. Fluctuation of free IAA in shoots (●) and roots (○) of *C. rubrum* during the inductive photoperiod 8 : $\overline{16}$ h. — B. Fluctuation of free IAA in shoots of *C. rubrum* (●) and *C. murale* (○) in the dark period (16 h) inductive for *C. rubrum*. — C. Fluctuation of free IAA in shoots of *C. rubrum* under conditions of prolonged darkness (●) and when darkness was preceded by photoperiod ($\overline{6}$: 8 h) which rephased the peak of endogenous rhythm of flowering (○). Arrows indicate the position of maxima of flowering rhythm in the former (full point with arrow) and the latter cases (open point with arrow).

TABLE 1
Time-course of 2-^{14}C-IAA uptake into cotyledons and leaves of 13 days old plants of *Chenopodium rubrum* [% of total amount]

Light [h]	Darkness [h]	Site of application leaves	cotyledons
3	—	38.4	10.4
—	2	—	3.5
—	4	—	6.4
3	6	53.3	15.6
—	13	—	16.2
3	13	55.8	26.6
3	22	62.5	35.6
—	24	—	37.6
—	45	—	53.5
3	45	79.6	52.2

to the receptor organs of photoperiodic signals in *C. rubrum*. The fluctuation pattern of free IAA in the roots of *C. rubrum* (exposed to photoperiodic conditions) and in the shoots of LDP *C. murale* are very similar to that found in the shoots of *C. rubrum* (Fig. 1A,B). Six hours darkness followed by eight hours light will shift the first peak in endogenous rhythm of flowering from the 13th to the 20th hour (KING and CUMMING 1972). This shift of phase in the endogenous rhythm brings about a corresponding displacement of IAA fluctuation as seen in Fig. 1C. However, this holds true only for the decreasing

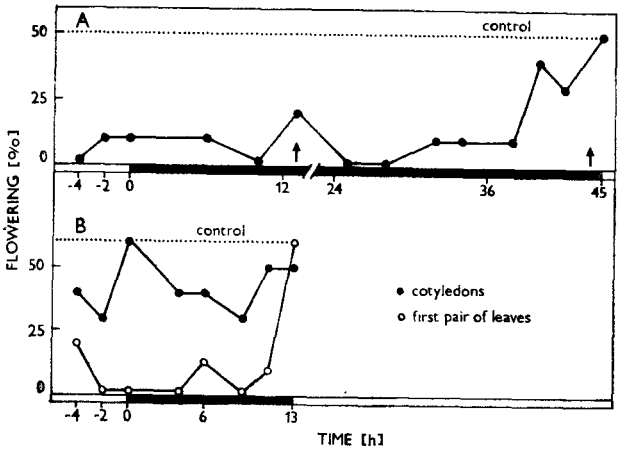

Fig. 2A. The effect of IAA (3 µl droplet of 3 . 10^{-4}M aqueous solution of IAA was applied to the cotyledons) on flowering of 6 days old *C. rubrum* plants. A single dose of IAA was applied before or during prolonged darkness and the changes of the magnitude of flowering estimated according to the second maximum of the endogenous rhythm of flowering. The position of the first and second maxima of flowering is indicated by the arrows.
Fig. 2B. The effect of IAA (3 µl droplet of 3 . 10^{-4}M aqueous solution of IAA was applied either to the first pair of leaves (○) or to the cotyledons (●)) on flowering of 14 days old *C. rubrum* plants. IAA was applied before or during inductive darkness and the changes of the magnitude of flowering were estimated according to the first maximum of endogenous rhythm of flowering (13th h).

phase of IAA level which becomes less abrupt. The character of the IAA rise after plants are transferred to darkness remains practically unchanged.

Timing of the Response to Inhibitory Action of Exogenous IAA in *C. rubrum*

To find out whether there are periods with different sensitivity to IAA application during inductive treatment, IAA was applied step-wise to plants

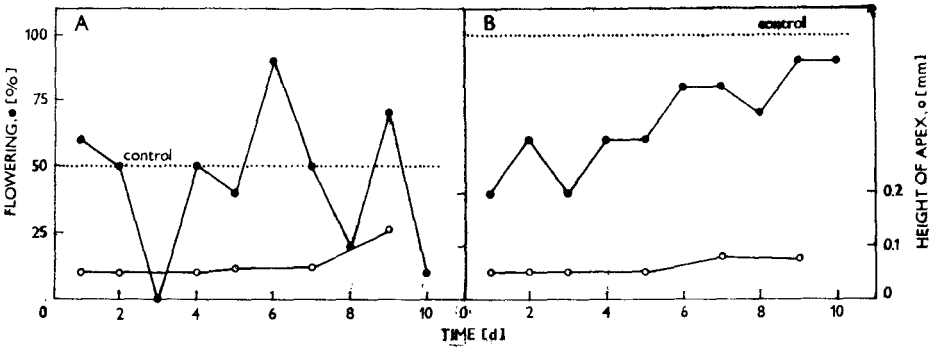

Fig. 3. The effect of IAA (3 μl droplet of 1 . 10^{-3}M aqueous solution was applied to the plumule) on flowering of 6 days old *C. murale* plants. IAA was applied on each consecutive day of 10 days induction by continuous illumination. The magnitude of flowering was estimated on the 5th day after induction. The height of the apical meristem (○) was evaluated at the moment of IAA application. A — plants grown with daily watering, B — plants grown with unlimited water supply.

shortly before or during the dark period. The effects were related to the first or to second peak of the endogenous rhythm of flowering. The results of an experiment with IAA application during 45 h of darkness are summarized in Fig. 2A, those with application within 13 dark hours in Fig. 2B. Applications close to the peaks of rhythm are distinctly less effective in inhibiting flowering than at other times. The application to leaves was more effective than that to cotyledons (Fig. 2B). Leaves were receptors for the photoperiodic signal at that time (unpublished results). The rate of ^{14}C —IAA uptake is illustrated in Table 1. There is much more uptake in light than in darkness and the uptake by cotyledons is less than by leaves.

Timing of the Response to Inhibitory Action of Exogenous IAA in *C. murale*

IAA application produced inhibition of flowering in *C. murale* (Fig. 3A). There exists a clear-cut sensitive phase to IAA application at the beginning of induction. Another inhibitory effect occurred at the end of induction, being possibly related to the growth rate of the plants. This is inferred from the fact that in plants with unlimited water supply and correspondingly high growth rate the second peak of inhibition is usually absent (Fig. 3B).

An Inhibitor of Ethylene Biosynthesis Reverts Flowering Inhibition Due to IAA

IAA treatment in four days old plants of *C. rubrum* induced to flower by 12 h dark period markedly decreased the percentage of flowering and the height of the shoot apical meristem. AVG applied simultaneously with auxin cancelled its effect to a great extent (Table 2). AVG alone is relatively inef-

TABLE 2

AVG effects on flowering of *Chenopodium rubrum* inhibited by IAA

Treatment	Time of treatment relative to the beginning of dark period [h]	Flowering [%]	Height of the shoot apex [mm]
Control	–	70	0.34 ± 0.015
5.10^{-4}M IAA	−2	0	0.11 ± 0.012
1.10^{-4}M AVG	−2	70	0.42 ± 0.042
5.10^{-4}M IAA + 1.10^{-4}M AVG	−2 −2	30	0.26 ± 0.023
5.10^{-4}M IAA	+12	10	0.24 ± 0.038
1.10^{-4}M AVG	+12	80	0.37 ± 0.032
5.10^{-4}M IAA + 1.10^{-4}M AVG	+12 +12	40	0.30 ± 0.03

Four days old plants were induced to flowering by one dark period of 12 h.

fective in changing the pattern of flowering. The results confirm a series of experiments made along this line by MACHÁČKOVÁ *et al.* (1985) on both *C. rubrum* and *C. murale*.

DISCUSSION

The similarity in the free IAA fluctuation in shoots and roots of *C. rubrum* and in shoots of *C. murale* and *C. rubrum* points to a general regulation of auxin level under photoperiodic conditions rather than to specific reflection of floral induction. However, the phylogenetic aspects of photoperiodism are at the moment too vague (*e.g.* SKRIPCHINSKIĬ 1975) to provide a sound basis for such considerations. This lack of "specific" response of free IAA to inductive conditions in shoots of *C. rubrum* is in accordance with the view of COOKE (1954), who held that changes in auxin level in leaves are not associated with flower induction. On the contrary, LOZHNIKOVA *et al.* (1982) has observed opposite tendencies in phytohormone fluctuation when comparing long-day and short-day species of tobacco. It may be argued that some physiological phenomena in flowering such as the fluctuation of IAA and rise in mitotic activity, are of general occurrence but exert their regulatory function only in target tissues and/or organs. Thus the previously established negative correlation between the capacity of flowering and low level of free IAA (TELTSCHEROVÁ *et al.* 1976, PAVLOVÁ and KREKULE 1984) may still be of physiological significance. The observed connection between change in position of the trough of free IAA level, and phase-shift of the endogenous rhythm of flowering points to such a possibility.

Periods of higher response to IAA which is inhibitory to flowering were found in both *C. rubrum* and in *C. murale*. The pattern observed in *C.*

rubrum suggests involvement of the endogenous rhythm of flowering with lower sensitivity to IAA occurring at the peaks of flowering capacity.

The floral effects of phytohormone (ABA) treatment within the endogenous rhythm of flowering have been studied in *C. rubrum* by ANDREAE (1976) and responsive periods were established. KING (1975) also found in *C. rubrum* a rhythmic response to GA_3 application, which was localized in the apex. Our results suggest that the effect is in both species localized in photoreceptive organs, *i.e.* cotyledons and leaves. Indirect evidence for this assumption is that organs receptive to photoperiodic conditions are also responsive to IAA (Fig. 3). Also, the degree of response to IAA is highest at the beginning of the rhythm in *C. rubrum* (*e.g.* application before the onset of the dark period) and decreases afterwards. The opposite should be true, when processes of evocation taking place in the apex after the arrival of stimulus from cotyledons and leaves (approximately 24 h after the beginning of the dark period), were also involved. The inhibition of flowering due to IAA effect found at the beginning of induction in *C. murale* is not likely to involve directly processes of apex growth and differentiation as these occur some four or five days later. Thus, besides the apical meristem being a site of direct inhibitory action of IAA (KREKULE and PŘÍVRATSKÝ 1974, PŘÍVRATSKÝ *et al.* 1976, SEIDLOVÁ and KHATOON 1976), auxin is likely to exert its effects in cotyledons and leaves, possibly interfering with the production of floral stimulus and affecting rhythmic phenomena of flowering. The effect of auxin in leaves has been already envisaged (*e.g.* SALISBURY 1955, NAKAYAMA 1958).

The existence of periods with higher response to IAA inhibition in LDP C. *murale* is rather surprising. Inhibition of flowering by auxin is usually considered to be a result of overall growth inhibition (LANG 1961) in long-day plants. This was certainly not the case here as various treatments did not cause difference in growth rate. Thus, it does not seem that inhibitory action of IAA in flowering is limited only to certain photoperiodic categories as has usually been claimed.

Serious problems are met when trying to correlate changes in responsiveness to IAA with the state of free IAA within the tissues. There is no marked fluctuation of IAA during the induction in *C. murale* (unpublished results). It is impossible at the moment to establish the level of IAA within the tissues which is critical for flowering inhibition. Moreover, the quantity of applied IAA exceeds by at least a double order of magnitude the range of fluctuation of endogenous free IAA. Even taking into account the rate of exogenous IAA uptake (Table 1) and its breakdown (unpublished results) it will still be several times higher. This problem should be further investigated.

AVG which inhibits a step leading to synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), a universal ethylene precursor (YANG and HOFFMAN 1984), reverted auxin inhibitory action. It was found that in *C. rubrum* auxin application brings about a rise in ethylene production which may in turn be cancelled by AVG (MACHÁČKOVÁ *et al.* in press). It may thus be assumed that ethylene represents an active factor in auxin inhibition of flowering. The data on ethrel (ethylene releasing compound) inhibitory effects on the flowering of *C. rubrum* (KHATOON *et al.* 1973) are in accordance with this assumption. The above results show the need for a revision of data obtained when studying auxin effects in flowering.

REFERENCES

AKSENOVA, N. P., BAVRINA, T. V., KONSTANTINOVA, T. N., CHAÏLAKHYAN, M. KH.: [Callus model of flowering and the perspectives of its investigation.] In Russ. — Zh. obshch. Biol. **33** : 523—538, 1972.

ANDREAE, S. R.: The effect of abscisic acid on the rhythmic flowering response of *Chenopodium rubrum*. — In: Abstracts XII International Botanical Congress. Vol. II. P. 277. Nauka, Leningrad 1975.

COOKE, A. R.: Changes in free auxin content during the photoinduction of short-day plants. — Plant Physiol. **29** : 440—444, 1954.

DOSTÁL, R., HOŠEK, M.: Über den Einfluss von Heteroauxin auf die Morphogenese bei *Circaea* (das Sachssche Phänomen). — Flora **131** : 263—286, 1937.

KHATOON, S., SEIDLOVÁ, F., KREKULE, J.: Time-dependence of auxin and ethrel effects on flowering in *Chenopodium rubrum* L. — Biol. Plant. **15** : 361—363, 1973.

KING, R. W.: Multiple circadian rhythms regulate photoperiodic flowering response in *Chenopodium rubrum*. — Can. J. Bot. **53** : 2631—2638, 1975.

KING, R. W., CUMMING, G.: Rhythms as photoperiodic timers in the control of flowering in *Chenopodium rubrum* L. — Planta **103** : 281—301, 1972.

KREKULE, J., PŘÍVRATSKÝ, J.: The shoot apex as the site of an inhibitory effect of applied auxin on photoperiodic induction of flowering in the short-day plant *Chenopodium rubrum* L. — Z. Pflanzenphysiol. **71** : 345—348, 1974.

LANG, A.: Auxins in flowering. — In: Ruhland, W. H. (ed.): Handbuch der Pflanzenphysiologie, Vol. XIV. Pp. 909—958. Springer Verlag, Berlin — Heidelberg — Göttingen 1961.

LOZHNIKOVA, V. N., KREKULE, J., SEIDLOVÁ, F., BAVRINA, T. V., CHAÏLAKHYAN, M. KH.: [Balance between gibberellins and abscisins in tobacco in the course of photoperiodic induction of flowering.] In Russ. — Fiziol. Rast. **29** : 247—252, 1982.

MACHÁČKOVÁ, I., KREKULE, J., SOUČKOVÁ, D. PŘIKRYL, Z., ULLMANN, J.,: Reversion of IAA-induced inhibition of flowering in long-day and short-day *Chenopodium* species by aminoethoxyvinyl glycine. — J. Plant Growth Regul. (in press).

MOUSDALE, D. M. A., BUTCHER, D. N., POWELL, R. G.: Spectrophotofluorimetric methods of determining indole-3-acetic acid. — In: HILLMAN, J. R., (ed.): Isolation of Plant Growth Substances. Pp. 27—39. London: Cambridge University Press, Cambridge 1978.

NAKAYAMA, S.: Studies on the dark process in the photoperiodic response of *Pharbitis* seedlings. — Sci. Rep. Tohoku Univ. **24** : 137—183, 1958.

PAVLOVÁ, L., KREKULE, J.: Fluctuation of free IAA under inductive and non-inductive photoperiods in *Chenopodium rubrum*. — Plant Growth Regulation **2** : 91—98, 1984.

PŘÍVRATSKÝ, J., TYKVA, R., KREKULE, J.: The possible role of auxin in reversing photoperiodic induction of flowering in a short-day plant *Chenopodium rubrum* L. — Z. Pflanzenphysiol. **78** : 377—386, 1976.

SALISBURY, F. B.: The dual role of auxin in flowering. — Plant Physiol. **30** : 327—334, 1955.

SEIDLOVÁ, F., KHATOON, S.: Effects of indol-3yl-acetic acid on floral induction and apical differentiation in *Chenopodium rubrum*. — Ann. Bot. **40** : 37—42, 1976.

SKRIPCHINSKIĬ, V. V.: Fotoperiodizm — Ego Proiskhozhdenie i Evolyutsiya. [Photoperiodism and Its Origin in Evolution.] — Nauka. Leningrad 1975.

TELTSCHEROVÁ, L., PAVLOVÁ, L., PLESKOTOVÁ, D.: Changes in the content of endogenous auxins in apical buds of *Chenopodium rubrum* L. induced with respect to the endogenous rhythm in capacity to flower. — Biol. Plant. **19** : 205—211, 1977.

ULLMANN, J., SEIDLOVÁ, F., KREKULE, J., PAVLOVÁ, L.: *Chenopodium rubrum* as a model plant for testing the flowering effects of PGRs. — Biol. Plant. **27** : 367—372, 1985.

VANDEN DRIESSCHE, T.: Circadian rhythmicity: general properties — as exemplified mainly by *Acetabularia* — and hypothesis on its cellular mechanism. — Arch. Biol. (Bruxelles) **91** : 49—76, 1980.

YANG, F. S., HOFFMAN, N. E.: Ethylene biosynthesis and its regulation in higher plants. — Annu. Rev. Plant Physiol, **35** : 155—189, 1984.