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Fluctuation of free IAA under inductive and non-inductive photoperiods in *Chenopodium rubrum*

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Abstract. Fluctuation in levels of endogenous free IAA has been followed in the SD plant *Chenopodium rubrum* under photoperiodic conditions inductive or not inductive of flowering. Endogenous IAA was measured fluorimetrically as α-pyrone. The level of IAA shows little fluctuation under continuous illumination. An endogenous rhythm of IAA fluctuation was found in plants transferred from light to continuous darkness, with a natural period of 30 hrs. The 'troughs' of minimum IAA level within the endogenous rhythm coincided with the peaks in the endogenous rhythm of flowering response, which possessed the same period length. The concentration of IAA in the shoot always decreased at the end of cycles of dark period that induce flowering. The results are discussed in relation to the role of IAA in flowering of SD plants.

Introduction

The role of auxin in flowering has been studied since the late 1930s. Hamner and Bonner [9] were among the first to report the inhibitory effect on flowering in Xanthium of IAA applied during the long dark period. These results were confirmed later by Green and Fuller [8], Hamner and Nanda [10] and by Leopold and Thimann [18]. In our model plant *Chenopodium rubrum,* auxins also cancel out short-day induction of flowering [12, 17].

Results from experiment involving exogenous applications of auxins led to investigations on the relationship between the photoperiodic conditions during flowering induction and the content of endogenous auxin [3, 4, 5, 7, 11, 15, 22, 23, 32], but no unequivocal relationship has so far been found. Teltscherová et al. [30] analysed the level of free IAA in shoot apices of *Chenopodium rubrum* as related to an endogenous rhythm of flowering. They found that the maximum of flowering corresponded to low levels of auxins and vice versa.

Diurnal fluctuations in auxin content as related to leaf movement was reported as early as 1941 [33]. Similarly Becker [1] found a rhythm of endogenous auxin in leaves of vegetative plants and in petals of *Kalanchoe blossfeldiana.*

Therefore further investigations were carried out to determine how far the photoperiodic conditions which either induce or do not induce flowering in *Chenopodium rubrum* might be reflected by changes in the level of free IAA.

Attention was also paid to the pattern of IAA fluctuation as related to the endogenous rhythm of flowering.

Materal and methods

Seedlings of the quantitative short day plant *Chenopodium rubrum* (selection 374) were used in all experiments. The length of the critical photoperiod for flowering in ecotype 374 is 16 hrs. The seeds of *Chenopodium rubrum* were germinated in distilled water according to the following schedule: 16 hrs illumination 7500 lx (fluorescent tubes) 30°C, 8 hrs darkness at 10°C and again 16 hrs illumination at 30°C. The seedlings were cultivated in half-strength Knop's solution in a small-volume growth chamber at 20°C under continuous illumination (day-light fluorescent tubes, 8000 lx at plant level). Photoperiodic regimes in all experiments started 5½ days after sowing. The plants were transferred back to continuous illumination after photoperiodic treatment and were later scored for the magnitude of flowering.

Endogenous IAA was estimated using the α-pyrone fluorimetric method [20, 21, 28]. Plant material was homogenized with solid CO_2 and extracted in darkness at −20°C for three hours using methanol with 0.02% sodium diethyldithiocarbamate (Lachema) as an antioxidant [19]. The water phase from the crude extract was further purified by solvent partitioning using dichloromethane. Alkali-labile conjugates were assayed after hydrolysis with 1 N NaOH at room temperature for one hour. The fluorescence of α-pyrone was measured using a Turner 111 fluorimeter equipped with filters No. 110816 and 110813 for excitation, and with Ilford filter 404 (Unicam) for emission.

Losses of IAA during extraction and purification were estimated by adding a known amount of ^{14}C-IAA as an internal standard. The radioactivity of the final sample was measured using a Mark 1 scintillation counter. An aliquot of the final sample was chromatographed on Silufol thin-layer plates (Kavalier Sázava), using the solvent system iso-propanol:ammonia:water (8:1:1), and the distribution of the radioactivity was analysed using a Friesecke-Hoepfner proportional counter.

Results

Experiment 1

Free IAA was estimated in cotyledons, apical buds and hypocotyls at the end of the following photoperiodic treatment, $\overline{16}$:8:$\overline{16}$ hours (the lines on the top indicate darkness), and compared with comparable data from plants grown under continuous illumination. An assumption was tested, that the nature of the changes in IAA content due to short days would be similar in all these organs, allowing the use of whole shoots in further investigations. At least 800 plants are required for the analysis, and these have to be sampled within rather a short period.

Table 1. The level of free IAA in various organs of 7-day-old *Chenopodium rubrum* seedlings (Expt. 1)

	Shoot	Apical bud	Cotyledon	Hypocotyl
Continuous illumination ng g^{-1} f.w.	53.5	232	19.2	113
% of total IAA in the shoot	100	37.2	26.6	36.2
$\overline{16}$:8:$\overline{16}$ ng g^{-1} f.w.	30.7	183.3	8.9	26.4
% of total IAA in the shoot	100	55.9	17.3	26.8

The results presented in Table 1 indicate that in both treatments the highest concentration of free IAA occurred in the apical buds and the lowest in the cotyledons. The content of free IAA at the end of second dark period was reduced in all parts of the plants receiving $\overline{16}$:8:$\overline{16}$. There was 80% of free IAA in the apical buds, 46% in the cotyledons and 27% in the hypocotyls as compared with the content of free IAA in the comparable parts of plants under continuous illumination (= 100%). The cotyledons and the young leaves were markedly shorter and the hypocotyls longer after two short days treatment, as compared to plants under continuous illumination.

Experiment 2

Plants were exposed to three 24 hr cycles of 20, 16, 12, 8 hrs photoperiods, to continuous light and to continuous darkness. The growth rate was estimated during this period, and shoots for IAA analysis were taken at two to three hours intervals. The time of collecting samples did not usually exceed 20 min, and the material was immediately frozen by addition of solid CO_2 and stored at $-20°C$. The growth rate of cotyledons and of the first and second leaf pair was inhibited, and the initiation of the third pair of leaves was retarded as compared with plants under continuous illumination. The growth of hypocotyl was considerably stimulated. The greatest growth effects were found in plants exposed to continuous darkness. Further details on the growth of *Chenopodium rubrum* seedlings under various photoperiodic regimes are given in [24, 31]. Examination of shoot apices under a stereo microscope did not reveal any morphological changes within the given period which might reflect the onset of the reproductive phase. It is normal for the activation of meristem growth to start in the course of the third cycle of an eight hour photoperiod [27].

The level of free IAA at age five and half days reached 54.3 ± 4.2 ng g^{-1} f.w. in the shoots. It increased slightly under continuous illumination, without any apparent fluctuation. Values of IAA content lay within the range 50–106 ng g^{-1} f.w., the maximum being found at the 49th hour.

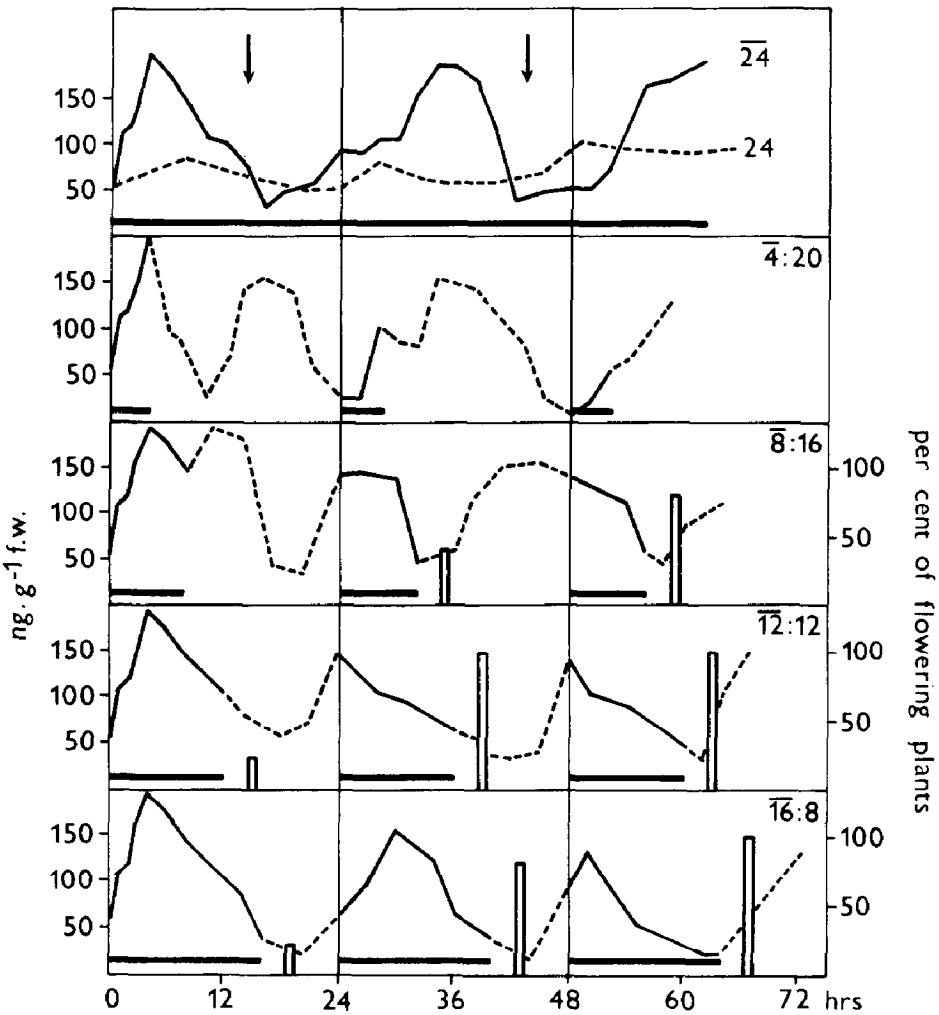

Figure 1. Level of free IAA in shoots of *Chenopodium rubrum* seedlings under different photoperiodic treatment. Full line – level of IAA in darkness, interrupted line – level of IAA in light. Photoperiodic regime is given by numbers indicating hours (line on the top of number represents darkness). Arrows indicate maxima of endogenous rhythm of flowering under continuous darkness. Columns reflect percentage of plants flowering due to respective photoperiodic treatment.

Transfer of plants to darkness resulted in an immediate rise in IAA level to 202 ± 18.3 ng g^{-1} f.w. during the first four hours. This increase was accompanied by a considerable drop in the content of alkali-labile IAA conjugates from 392 ng g^{-1} f.w. under continuous illumination to 220 ng g^{-1} f.w. in darkness. The level of free IAA then dropped to minimum (31.7 ± 3 ng g^{-1} f.w.) after 16 hrs in plants maintained in darkness. Fluctuation of IAA levels

thereafter exhibited a rhythmic pattern with a period length of 30 hrs Figure 1,

IAA fluctuated according to the length of the photoperiod. Dark periods not exceeding four hours brought about the ascending phase of the rhythm of IAA fluctuation, whereas dark periods longer than eight hours (which possess an inductive character) resulted in its descending phase.

Discussion

Our findings have shown that fluctuation in the level of free IAA in *Chenopodium rubrum* has an endogenous character, its pattern depending on the length of photoperiod. These results are in conformity with the data of Bezler and Bünning [2] on the endogenous rhythm of IAA changes in soybean cv. Peking (SDP) and in *Hyoscyamus* (LDP), and with those obtained by Becker [1] in *Kalanchoe.*

The existence of diurnal changes in IAA content may explain some of the discrepancies found in the literature. Most authors did not take account of rhythmicity except that in some cases samples were taken at the same time of the day. Such methods may clearly lead to erroneous results, especially when changes in IAA within the first days following induction are being investigated. Maximum and minimum IAA levels are often shifted by changes in photoperiodic schedule, and differ from further photoperiodic cycles [13]. Kiyosawa and Wake [14] investigated rhythmicity of endogenous IAA fluctuation in varieties of soybean and came to following conclusion: 'Measurement performed once a day may have not any effective mean to determine the effect of day length on auxin'. The same conclusion may be reached by analyzing the data for endogenous auxin within 15 inductive cycles in the LDP *Hyoscyamus niger* obtained by Kopcewicz et al. [16].

The results of experiment 1, as well as the data of Kiyosawa and Wake [14], substantiate the view that the fluctuation of free IAA exhibits the same or a closely similar pattern in all above-ground organs of the plant. Considerable changes were found in free IAA level in both the cotyledons and hypocotyls as compared with plants under continuous illumination. The range of amplitude of IAA changes in apical buds is difficult to evaluate without establishing the maximal value of IAA. Kiyosawa and Wake [14] found a lower amplitude in the apical bud as compared with the leaves.

Two sites of auxin involvement in flower initiation have to be considered, as indicated already by Salisbury [26]. Auxin may play a role either in the photoperiodic induction taking place in the leaves (cotyledons in our case), or in the evocation [6] of the apical meristem. It was found that the maxima of the endogenous rhythm of flowering in *Chenopodium rubrum* [29] coincide with the minima of the endogenous rhythm of IAA fluctuation. We may therefore assume that the endogenous rhythm of flowering (whether an oscillation in the capacity for flowering or a fluctuation in the production of

a flowering stimulus) and the rhythm of free IAA fluctuation exhibit an opposite pattern of phasing. The period length is the same in both rhythms. The existence of a negative correlation between the level of free IAA and the capacity to flower is also seen when the concentration of IAA under inductive and non-inductive photoperiods is compared. The level of IAA always decreases at the end of the dark period of an inductive cycle. Similarly, when the effect of inductive dark period is cancelled by a light break (irradiation at 660 nm), a concomitant rise in the level of free IAA is at once observed [25].

This data, although in accord with the general view on the detrimental effect of auxin on flowering of SD plants, does not tell us if the relationship between auxin and flower initiation is causal in nature and/or is used in coincidence. The amplitude of IAA fluctuation in various organs, e.g. in hypocotyls and cotyledons, might perhaps reflect changes in growth rate. Thus, the drop in auxin content in hypcotyls may be due to their marked elongation.

It was found in experiment 1 that the level of free IAA in the apical bud is considerably higher than in the cotyledons. Thus, the evocation of the apical meristem takes place at a considerably higher level of IAA than the synthesis of the floral stimulus in the cotyledons, providing the content of IAA in young leaves, which represent the substantial part of the apical bud, and in the apical meristem is similar enough. On the other hand it follows from the experiments of Krekule and Přívratský [17], and by Khatoon and Seidlová [12] that evocation in *Chenopodium* is more responsive to the inhibitory effect of applied auxin than is photoperiodic induction in the cotyledons.

Despite various problems in interpreting these results in relation to flowering, there is good reason for further studies of the role of auxin in photoperiodic flower induction.

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