BIOLOGIA PLANTARUM (PRAHA)

33 (1) : 26–31, 1991

The Role of Indol-3-ylacetic Acid in Regulation of Juvenility in *Xanthium strumarium* L.

V. Z. PODOLNYI*, IVANA MACHÁČKOVÁ**, ZUZANA JOSEFUSOVÁ-VONDRÁKOVÁ**, J. EDER**, J. KREKULE** and M. Kh. CHAILAKHYAN*

*Institute of Plant Physiology of A. K. Timiryazev, Academy of Sciences of the USSR, Botanicheskaya 35, 127276 Moskva, USSR and
**Institute of Experimental Botany, Czechoslovak Academy of Sciences, Ke dvoru 15, 166 30 Praha 6, Czechoslovakia

Abstract. Cotyledons of *Xanthium strumarium*, organs with low sensitivity to photoperiodic treatment show a higher free indol-3-ylacetic acid level (by about 35 %) than the first pair leaves, organs with high sensitivity to photoperiodic treatment. This was seen in plants of three different age groups: A. with the first pair of leaves of 15–20 mm in length; B. with the first pair of leaves having finished their growth and C. with the third leaf of 30–40 mm in length.

Changes in free IAA level during the inductive dark period were similar in both cotyledons and leaves of the first pair. The level of IAA rose in the first half of the dark period, began to decrease in the latter half, reaching nearly initial level at its end.

Application of IAA (10^{-4} – 10^{-6}M) to the cotyledons reduced their already low photoperiodic sensitivity resulting in inhibition of flowering (almost 70 % using 10^{-4}M IAA).

Elevated free IAA level is assumed to be one of the causal factors of low photoperiodic sensitivity of cotyledons.

Developing cotyledons of *Xanthium strumarium* differ sharply in their photoperiodic sensitivity from the leaves of the first pair. Cotyledons are not only much less sensitive than the first pair of leaves, but they even can inhibit flowering induced *via* leaves or induce proliferation of already induced primordia of male inflorescence (Chailakhyan and Podolnyi 1968, Podolnyi and Chailakhyan 1970, Podolnyi *et al.* 1986). It was suggested on the basis of these results that low photoperiodic sensitivity of cotyledons was due to a high level of inhibitors of flowering.

Several compounds were shown to inhibit flowering. For example, gibberellic acid was able to turn *Hedera helix* back from the adult to the juvenile state (Robbins

Received December 22, 1989; accepted March 16, 1990
Reprint requests should be addressed to I. Macháčková.

1957). In several short-day plants, including *Xanthium strumarium*, increased IAA level might be the cause of flowering inhibition (Lang 1961, Jacobs 1985). Thus, it is possible that IAA takes part in sustaining the juvenile state in *X. strumarium*. To test this potential role of IAA, we compared the free IAA level in cotyledons and leaves of the first pair, determined its changes during the inductive dark period and in addition also the effect of exogenous IAA on photoperiodic sensitivity of cotyledons.

MATERIAL AND METHODS

Plants of *Xanthium strumarium* L. were grown in pots in soil at 20 ± 2 °C under continuous light (fluorescent tubes, 35 Wm^{-2}). Free IAA level was determined in three developmental phases: when

A – cotyledons were fully opened and leaves of the first pair were about 15–20 mm long;
B – leaves of the first pair reached the final stage of growth;
C – the third leaf was about 30–40 mm long.
The interval from A to B was 4 days, from B to C 6 d.

Changes in the free IAA level during one inductive dark period of 16 h were studied in 10-d-old plants, with the first pair of leaves just having finished their growth and the third leaf about 12–20 mm long. In addition to cotyledons and leaves, we analyzed also the apical parts (including the third and following leaves). Material for analyses was taken at the beginning, in the middle (8 h) and at the end (16 h) of the dark period. Control plants were kept under continuous illumination.

Free IAA level was determined in fresh material after its homogenization in liquid nitrogen. Extraction was performed by 80 % aqueous methanol with 100 mg l^{-1} of butylated hydroxytoluene (10 ml g^{-1} FW) at 4 °C and repeated once. Further purification and final determination by means of HPLC with fluorimetric detection was recently described in detail (Eder *et al.* 1988, Lozhnikova *et al.* in press).

The effect of exogenous IAA was studied in plants in developmental stage A, in which all leaves were removed at the beginning and also during the experiment, leaving only the cotyledons. These plants were subjected to 10 inductive cycles of 16 h darkness, 8 h light; IAA was applied to the cotyledons every other day in concentrations 10^{-6}, 10^{-5} and 10^{-4} M. Plants were scored for flowering percentage and apex height on the 10th day using a stereomicroscope. The experiment was repeated five times, each treatment comprising 10–15 plants. The results in Table 2 are from one representative experiment.

RESULTS AND DISCUSSION

Free IAA Level in Cotyledons and First Pair of Leaves

In vegetative plants cotyledons had a higher free IAA level than the leaves of the first pair (Table 1). This was true in both developmental stages tested – when the leaves of the first pair had just finished their growth and later, when IAA level in both organs had already decreased. This decrease was more pronounced in leaves (Table 1).

Correlation of the higher free IAA level in cotyledons with their low photoperiodic sensitivity shown earlier (Chailakhyan and Podolnyi 1968, Podolnyi and Chailakhyan 1970) suggests that higher IAA level might be one of the causal agents in low photoperiodic sensitivity of *X. strumarium* cotyledons. The possibility of an inhibitory action of IAA in receptor organs of photoperiodic treatment was already suggested (Jacobs 1985). Fluctuations in free IAA level during different photoperiods were observed in another short-day plant, *Chenopodium rubrum* in which decreases in the IAA level coincided with increases in the capacity for flowering (Pavlová and Krekule 1984). Such a correlation was sought also in *X. strumarium*: we observed the changes in free IAA level in cotyledons, the first pair of leaves and the apical parts during the inductive dark period (IDP). In both organs – cotyledons and leaves – the IAA level increased significantly toward the middle of IDP, in cotyledons' almost 2.6fold and in leaves 4.2fold. Thereafter the IAA level decreased again, reaching the original level at the end of IDP (Fig. 1). However, in the apical part free IAA level was slowly decreasing throughout IDP. Control plants under continuous light showed no significant changes in free IAA level (Fig. 1).

The similarity of changes in free IAA level in organs with low or high photoperiodic sensitivity seem to indicate that these changes are more directly coupled with changes in the photoperiodic regime than with "floral stimulus" production as already suggested by Macháčková *et al.* (1988) for *Chenopodium* species. They observed the same character of changes in free IAA level due to the same

TABLE 1

Free IAA level in cotyledons and leaves of the first pair of vegetative plants of *Xanthium strumarium*

Developmental stage	Free IAA [ng g^{-1} (f.m.)]	
	Leaves of the first pair	Cotyledons
A (first leaves 15–20 mm)	–	19.6
B (first leaves finished growth)	21.3	29.6
C (third leaf 30–40 mm)	6.8	11.7

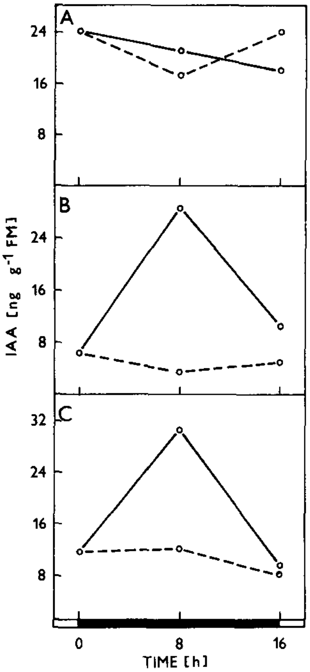

Fig. 1. Changes in free IAA level during the inductive dark period of 16 h in apical parts (A), leaves of the first pair (B) and cotyledons (C) in 10-d-old *Xanthium strumarium* plants. – Full line : induced plants; dashed line: control plants (kept in continuous light).

photoperiod in both short-day *C. rubrum* and long-day *C. murale*, irrespective of the flowering behaviour of these species.

The Effect of IAA Application on Photoperiodic Sensitivity of Cotyledons

If IAA has any role in the regulation of photoperiodic sensitivity of receptor organs, then IAA application must exert some effect on it. As shown by Table 2, this was the case. Exogenous IAA decreased the sensitivity of cotyledons resulting in the reduction of the number of flowering plants. This inhibitory action increased with the IAA concentration used (Table 2). Apart from the effect on flowering, lower IAA concentrations (10^{-6} and 10^{-5} M) had no effect on plant growth and habitus. The highest IAA concentration used (10^{-4} M) brought about hypocotyl curvature and, hyponasty of cotyledons. These changes seem to be attributable to increased ethylene production which was extensively described after IAA application (Yang and Hoffman 1984).

If we summarize the present results, studies of the endogenous level of free IAA in cotyledons and leaves of the first pair in different developmental phases and its changes during the IDP, together with the inhibitory effect of applied IAA on

TABLE 2

The effect of IAA application on flowering of *Xanthium strumarium* seedlings having only cotyledons

	IAA concentration [M]			
	0	10^{-6}	10^{-5}	10^{-4}
Flowering [%]	75.0	58.3	16.0	8.3
Apex height [mm]	0.29 ± 0.03	0.30 ± 0.01	0.30 ± 0.01	0.26 ± 0.03

photoperiodic sensitivity of cotyledons, allow us to assume that increased IAA level in cotyledons may be important to maintaining their juvenile character. Further, the presented results, together with those for *C. rubrum* and *C. murale* (Krekule *et al.* 1985), show that one of the inhibitory effects of IAA on flowering may be localized in cotyledons. Cotyledons were shown to be the perception organ for the photoperiodic signal in young *C. rubrum* plants (King and Cumming 1972). It is thus possible that IAA interferes either with perception or realization of the photoperiodic signal. Explants of *Streptocarpus nobilis* (Simmonds 1987) could be induced in the presence of IAA, but in spite of this already induced explants did not flower in IAA presence. Therefore, he considered IAA an inhibitor of the expression of the induced state.

The shoot apex of *C. rubrum* was shown to be another site of IAA inhibitory action on flowering (Seidlová and Khatoon 1976, Krekule *et al.* 1989). We cannot exclude that part of the IAA applied to cotyledons was transported to the apex and exerted its effect there possibly by strengthening apical dominance (Krekule and Přívratský 1976, Seidlová and Khatoon 1976).

On the other hand, a similar pattern of changes in free IAA level during IDP in both cotyledons and leaves, despite the significant difference in their photoperiodic sensitivity and the degree of juvenility may indicate that IAA is not the only factor regulating juvenility. Other hormones are assumed to be capable of taking part in juvenility regulation: decreased ABA level (Podolnyi *et al.* 1989) and increased cytokinin level (Podolnyi *et al.* in press) in cotyledons were shown to sustain their juvenile character.

REFERENCES

Chailakhyan, M. Kh., Podolnyi, V. Z.: [Role of cotyledons in the juvenile period of plant development.] In Russ. – Fiziol. Rast. **15**: 949–957, 1968.

Eder, J., Rovenská, J., Kutáček, M., Čermák, V.: HPLC analysis of indole compounds in *Agrobacterium* and transformed tobacco cells. – In: Kutáček, M., Bandurski, R. S., Krekule, J. (ed.): Physiology and Biochemistry of Auxins in Plants. (Proc. Int. Symp. Liblice, Czechoslovakia 1987.) Pp. 389–390. Academia, Praha; SPB Acad. Publ., The Hague 1988.

Jacobs, W. P.: The role of auxin in inductive phenomena. – Biol. Plant. **27**: 303–309, 1985.

King, R. W., Cumming, B. G.: The role of phytochrome in photoperiodic time measurement and its relation to rhythmic timekeeping in the control of flowering in *Chenopodium rubrum*. – Planta **108**: 39–57, 1972.

Krekule, J., Macháčková, I., Pavlová, L., Seidlová, F.: Hormonal signals in photoperiodic control of flower initiation. – In: Krekule, J., Seidlová, F. (ed.): Signals in Plant Development. Pp. 145–162. SPB Acad. Publ., The Hague 1989.

Krekule, J., Pavlová, L., Součková, D., Macháčková, I.: Auxin in flowering of short-day and long-day *Chenopodium* species. – Biol. Plant. **27**: 310–317, 1985.

Krekule, J., Přívratský, J.: An attempt to distinguish between inductive and postinductive effects of short-day plants. – Acta Univ. N. Copernici, Biol. **18**: 45–51, 1976.

Lang, A.: Auxins in flowering. – Encycl. Plant Physiol. **14**: 909–950, 1961.

Macháčková, I., Krekule, J., Ullmann, J., Pavlová, L., Součková, D.: Photoperiodic control of auxin and ethylene level in flowering in *Chenopodium* sp. – In: Kutáček, M., Bandurski, R. S., Krekule, J. (ed.): Physiology and Biochemistry of Auxins in Plants (Proc. Int. Symp. Liblice, Czechoslovakia 1987.) Pp. 319–323, Academia, Praha; SPB Acad. Publ., The Hague 1988.

Pavlová, L., Krekule, J.: Fluctuation of free IAA under inductive and non-inductive photoperiods in *Chenopodium rubrum*. – Plant Growth Regul. **2**: 91–98, 1984.

Podolnyi, V. Z., Chailakhyan, M. Kh.: [Inhibition of shoots flowering by coclebur cotyledons.] In Russ. – Fiziol. Rast. **17** : 502–506, 1970.

Podolnyi, V. Z., Khmelnitskaya, I. Ph., Chailakhyan, M. Kh.: [Ability of *Xanthium strumarium* cotyledons to induce proliferation of apical inflorescence.] In Russ. – Dokl. Akad. Nauk SSSR **291**: 765–769, 1986.

Podolnyi, V. Z., Josefusová, Z., Khmelnitskaya, I. Ph., Verenchikov, S. P., Krekule, J., Chailakhyan, M. Kh.: Abscisic acid as a potent regulator of the transition from juvenile to mature stage in *Xanthium strumarium*. – Biol. Plant. **31**: 139–144, 1989.

Robbins, W. J.: Gibberellic acid and reversal of adult *Hedera* to juvenile state. – Amer. J. Bot. **44**: 743–746, 1957.

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

Simmonds, J.: IAA inhibition of *in vitro* flowering of the short-day plant *Streptocarpus nobilis*. An effect on maintenance of induction. – J. Plant Physiol. **131**: 191–199, 1987.

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