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Reversal of IAA-Induced Inhibition of Flowering by Aminoethoxyvinylglycine in *Chenopodium*

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Abstract. Aminoethoxyvinylglycine (AVG) applied as a droplet (3 μl, 0.1 mM) to the plumule of seedlings of both the short-day plant *Chenopodium rubrum* and the long-day plant *Chenopodium murale* counteracted to a great extent or even canceled the inhibition of flowering due to exogenous indole-3-acetic acid (IAA). This effect was more pronounced with the two substances administered simultaneously than with later application of AVG alone. AVG by itself in some cases promoted the percentage of flowering in both *Chenopodium* species. Application of IAA to the shoot apex was shown to elevate ethylene production in both species, whereas application of AVG alone was shown to suppress it. Thus, ethylene may be considered an active agent of flowering inhibition brought about by IAA application.

Application of auxins is known to elevate ethylene production in plants (Yang and Hoffman 1984, and references therein). On the basis of this knowledge it has been shown that some effects of auxin are in fact due to ethylene—e.g., inhibition of root growth (Mulkey et al. 1982) and auxin-induced epinasty (Amrhein and Schneebeck 1980). The role of auxin in regulating apical dominance also seems to be mediated at least partly by ethylene (Blake et al. 1983).

In our previous work we have found that IAA application to the apical bud of the short-day plant (SDP) *Chenopodium rubrum* cancels photoperiodic flower induction (Krekule and Přívratský 1974, Přívratský et al. 1976). This effect is localized in the apical meristem and involves the inhibition of axillary sites (Seidlová and Khatoon 1976). A similar pattern of flowering inhibition was observed after ethephon (2-chloroethyl phosphonic acid) application (Khatoon et al. 1973). Moreover, IAA inhibited flowering in the long-day plant (LDP) *Chenopodium murale* (Krekule et al. 1985). Thus, it is interesting to

investigate whether the inhibitory effect of auxin on photoperiodically induced flowering is mediated by ethylene. We aimed to test this hypothesis by means of AVG, an inhibitor of ethylene sysnthesis (Lieberman 1979).

Materials and Methods

Materials

Seedlings of the quantitative SDP *Chenopodium rubrum* (selection 374) and the quantitative LDP *Chenopodium murale* (selection 197) were used.

Plant Cultivation

The detailed schemes used for germination and cultivation of *Ch. rubrum* have been recently described (Ullmann et al. 1985). Both species were cultivated in a small volume growth chambers at 20 ± 1°C, with illumination provided by fluorescent tubes (8000 lx at plant level). The seedlings were grown in half-strength Knop's solution—*Ch. rubrum* as hydroponics, *Ch. murale* in perlite.

The inductive conditions (2 photoperiods) were chosen in *Ch. rubrum* to bring about different percentages of flowering. Thus, the length of dark period in the first inductive cycle ranged from optimal (12 h) to below critical (9 or 6 h) (Ullmann et al. 1985).

Ch. murale seedlings were used at the ages of 6 and 13 days and were induced to flower by 8 days of continuous light. Before and after induction the seedlings were cultivated under continuous illumination (*Ch. rubrum*) and under short-day (8 h) light—*Ch. murale*.

Application of IAA, AVG, and Ethephon

IAA (0.5 mM), AVG (0.1 mM) and ethephon (0.3 mM) were applied as a 3-μl droplet of water solution to the plumule. The treatment took place 2 h before the second inductive dark period in *Ch. rubrum* and on the first (for 6-day-old plants) and fourth (for 13-day-old plants) days of induction in *Ch. murale* (Krekule et al. 1985). Ten plants for each treatment were dissected using a stereomicroscope and scored for percentage of plants flowering and height of the apical meristem (7 days after the end of induction).

Ethylene Determination

Ethylene production by *Ch. rubrum* and *Ch. murale* seedlings as affected by IAA and AVG was followed in sealed Plexiglas boxes, stoppered with a rubber septum, and incubated for 2–3 h in light at 20 ± 1°C. IAA (0.5 mM) and AVG (0.1 mM) were applied as 3-μl droplets to the apex of each plant (50 plants per box) 1 h before sealing the boxes. At the end of incubation a 5-ml sample of the atmosphere was withdrawn with a syringe, and the ethylene content was

Table 1. Effects of IAA, AVG, and ethephon on flowering of *Chenopodium rubrum*. Five-day-old plants were induced to flower.

Treatment	Time of treatment related to beginning of 2nd dark period (h)	Apex height (mm) ± SE	Flowering (%)
A: 6 h dark, 12 h light, 12 h dark			
Control	—	0.195 ± 0.017	0
5.10^{-4} M IAA	−2	0.115 ± 0.008**	0
10^{-4} M AVG	−2	0.180 ± 0.023	20
5.10^{-4} M IAA + 10^{-4} M AVG	−2 −2	0.150 ± 0.011*	10
5.10^{-4} M IAA + 10^{-4} M AVG	−2 +4	0.135 ± 0.012	0
3.10^{-4} M ethephon	−2	0.128 ± 0.017**	0
B: 9 h dark, 12 h light, 12 h dark			
Control	—	0.255 ± 0.016	20
5.10^{-4} M IAA	−2	0.155 ± 0.017**	0
10^{-4} M AVG	−2	0.240 ± 0.026	30
5.10^{-4} M IAA + 10^{-4} M AVG	−2 −2	0.225 ± 0.015**	30
5.10^{-4} M IAA + 10^{-4} M AVG	−2 +4	0.190 ± 0.022*	20
3.10^{-4} M ethephon	−2	0.080 ± 0.011***	0
C: 12 h dark, 12 h light, 12 h dark			
Control	—	0.325 ± 0.025	60
5.10^{-4} M IAA	−2	0.150 ± 0.022***	20
10^{-4} M AVG	−2	0.330 ± 0.025	60
5.10^{-4} M IAA + 10^{-4} M AVG	−2 −2	0.240 ± 0.042*	50
5.10^{-4} M IAA + 10^{-4} M AVG	−2 +4	0.205 ± 0.016*	30
3.10^{-4} M ethephon	−2	0.295 ± 0.012**	20

Significantly different from the control at $^{**}p = 0.01$ and $^{***}p = 0.001$.
Significantly different from IAA treatment at $^{*}p = 0.05$ and $^{**}p = 0.01$.

determined on a gas chromatograph (Perkin-Elmer F-21) equipped with Porapak N column and flame-ionization detector.

Results

AVG Effects in Ch. rubrum

The application of 0.5 mM IAA to the plumule of *Ch. rubrum* plantlets 2 h before the second inductive dark period brought about inhibition of flowering and/or of the growth of the apical bud in all three photoperiodic treatments (Table 1). AVG (0.1 mM) counteracted the inhibitory action of IAA when applied concomitantly, the percentage of flowering sometimes even exceeding the control values. The inhibition of apex growth was in most cases counter-

Table 2. Effects of IAA, AVG, and ethephon on flowering of *Chenopodium murale*. Six- (A) and 13- (B) day-old plants were induced to flower by 8 days of continuous illumination.

Treatment	Time of treatment (day of induction)	Apex height (mm) ± SE	Flowering (%)
A: 6-day-old plants			
Control	—	0.105 ± 0.012	10
5.10^{-4} M IAA	1	0.050 ± 0.000***	0
10^{-4} M AVG	1	0.107 ± 0.011	0
5.10^{-4} M IAA + 10^{-4} M AVG	1 1	0.105 ± 0.009**	10
5.10^{-4} M IAA + 10^{-4} M AVG	1 (5 h later)	0.095 ± 0.000**	10
B: 13-day-old plants			
Control	—	0.145 ± 0.024	40
5.10^{-4} M IAA	4	0.105 ± 0.025	0
10^{-4} M AVG	4	0.155 ± 0.020	50
5.10^{-4} M IAA + 10^{-4} M AVG	4 4	0.170 ± 0.015*	50
5.10^{-4} M IAA + 10^{-4} M AVG	4 (5 h later)	0.155 ± 0.016*	30
3.10^{-4} M ethephon	4	0.070 ± 0.011**	0

Significantly different from the control at $^{**}p = 0.01$ and $^{***}p = 0.001$.
Significantly different from IAA treatment at $^{*}p = 0.05$ and $^{**}p = 0.01$.

acted only partly. If AVG was applied later (6 h and later after IAA), its effect was less pronounced (Table 1). AVG applied by itself increased the percentage of flowering in some cases.

AVG Effects in Ch. murale

In *Ch. murale* auxin also inhibited flower induction: in 6-day-old plants when applied on the first day of induction, and in 13-day-old plants when applied on the fourth day (Table 2). Moreover, AVG applied at the same time again counteracted the inhibitory auxin effect on flower induction and apex height. AVG applied later (5 h after IAA) was a little less effective, while alone it slightly stimulated apex height and flowering in the older plants only.

Inhibition of flowering by ethephon

In both species ethephon (0.3 mM) applied to the plumule (the time of application was the same as for IAA) had a similar inhibitory effect as IAA (Tables 1, 2).

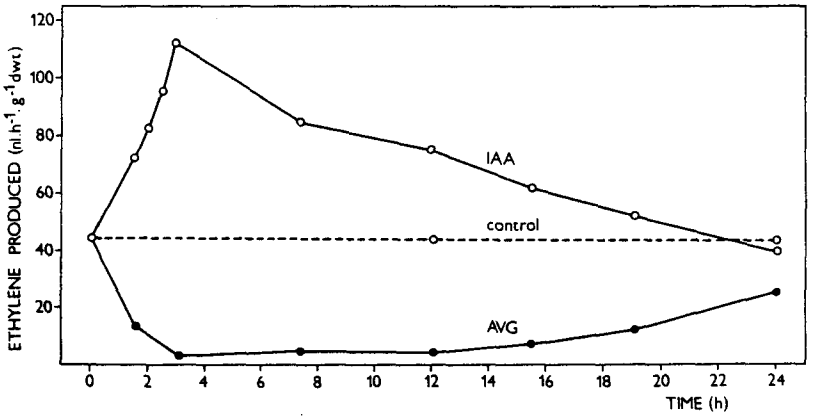

Fig. 1. The effect of 0.5 mM IAA (O——O) and 0.1 mM AVG (●——●) on ethylene production by 5-day-old *Chenopodium rubrum* plants in continuous light. (O- - -O, Control.)

Effects of IAA and AVG on Ethylene Production

IAA (0.5 mM) stimulated ethylene formation in both species. The effect was measurable from 90 min after IAA application, and the greatest level of stimulation (253% of control) was observed after 3 h. This was then followed by a slow decrease of IAA-induced ethylene production until after 24 h, when ethylene production was equal to the control. Figure 1 shows typical results for *Ch. rubrum*. Concentration dependence of IAA-induced ethylene production is shown in Fig. 2. Concentration as low as 10^{-7} M enhanced ethylene release slightly. The most effective IAA concentration is 0.5 mM; that of 1 mM is supraoptimal. We found higher ethylene production in darkness than in light, and the stimulatory effects of darkness and IAA were additive (Fig. 2). AVG at 0.1 mM almost completely blocked ethylene production in both *Chenopodium* species. Its effect lasted 12 h and then slowly declined, such that after 24 h ethylene production was 55% of the control (Fig. 1).

Discussion

We have shown that AVG partly counteracted or even canceled IAA-induced inhibition of flowering, both in the SDP *Ch. rubrum* and in the LDP *Ch. murale*. Moreover, treatment with ethephon, an ethylene-releasing compound, resulted in similar effects on flowering in *Ch. rubrum* as those of IAA, as already reported by Khatoon et al. (1973). Our present results show marked inhibition of flowering by ethephon also in *Ch. murale*. All of these effects support the view that the inhibition of flowering brought about by exogenous IAA is in fact due to increased ethylene levels. Auxin-induced ethylene synthesis represents a rather common phenomenon in plants (see Yang and Hoffman 1984 for a review). Such an interpretation is further corroborated by the discovery of increased ethylene production in *Ch. rubrum* and *Ch. murale* following IAA application and its suppression by AVG.

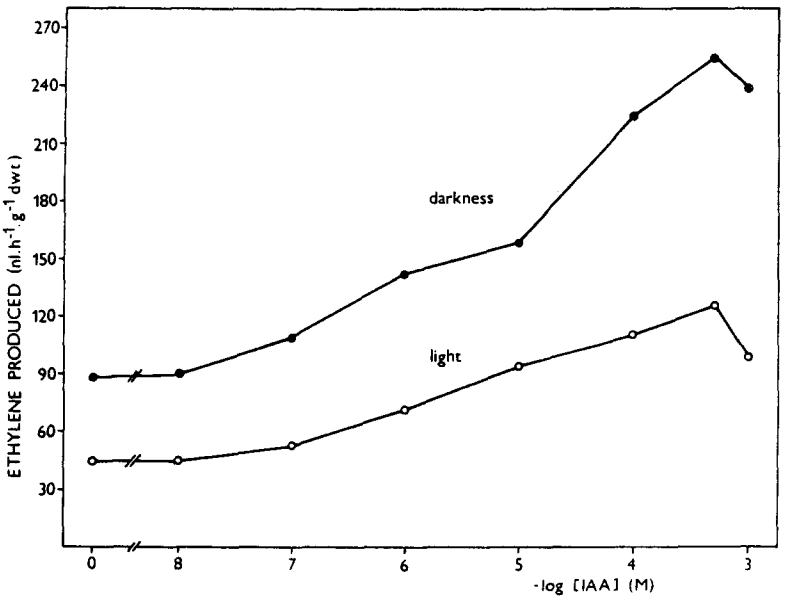

Fig. 2. The effect of IAA concentration on ethylene production by 5-day-old *Chenopodium rubrum* plants in light (○——○) and darkness (●——●).

However, it has also been found that lower IAA concentrations (10^{-5} M to 10^{-7} M), which do not affect flowering, elevate ethylene production, although to a lesser extent. It is therefore likely that ethylene has to be present at a certain level to inhibit flowering. This assumption is further supported by the finding that low ethephon concentrations (0.1 mM and below) are ineffective in flowering inhibition (unpublished results).

Although multiple sites of auxin action in flowering may exist in *Chenopodium* (Krekule et al. 1985), the most common of these seems to be localized at the apex (Přívratský et al. 1976). Axillary buds represent one target for auxin action in such a case (Seidlová and Khatoon 1976). The observation that auxin and ethephon exert the same response (Khatoon et al. 1973) points to a similarity in the sites of their action. The fact that in some cases (e.g., in weakly induced *Ch. rubrum* and in older plants of *Ch. murale*) AVG promoted flowering and branching of the apex as well as shoot growth (data not given) supports such an assumption.

It is generally agreed that auxin affects LD and SD plants in different ways, promoting flowering in the former and suppressing it in the latter (e.g., Lang 1961). However, the similarity of auxin action in flowering of LD and SD *Chenopodium* was observed by Krekule et al. (1985), and analogous patterns of free auxin level fluctuation were also established in both cases. Thus the similarity between AVG effects in both photoperiodic types of *Chenopodium* is in accordance with the above data.

We may conclude that ethylene mediates the IAA-induced inhibition of flowering in SD and LD *Chenopodium* species. Some evidence points to the pos-

sibility that endogenous ethylene might regulate flowering, but further research is necessary to confirm this idea.

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