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Ethylene Production and Metabolism of 1-Aminocyclopropane-1-Carboxylic Acid in *Chenopodium rubrum L.* as Influenced by Photoperiodic Flower Induction

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Abstract. *Chenopodium rubrum* plants, induced to flower by three cycles of 12 h darkness and 12 h light, produced 42% less ethylene than vegetative plants kept under continuous light. Plants that had each dark cycle broken by 2 h light in the middle did not flower and produced almost as much ethylene as the vegetative plants. Shoots and roots of plants of all three experimental treatments had a similar content of 1-aminocyclopropane-1-carboxylic acid (ACC), the mean amounting to about 2 nmol · g^{-1} dry weight. Also the content of N-malonyl-ACC (MACC) was similar in shoots of all three treatments. MACC content in roots was shown to be much higher, especially in the treatments with three dark periods (about 85 nmol · g^{-1} dry weight). When labeled [2,3-^{14}C] ACC was administered, the relative contents of ACC and MACC were very similar among all three treatments. The only process influenced by flower induction was ACC conversion to ethylene. Induced plants converted 36% less ACC than the vegetative ones. Plants subjected to night-break converted almost as much ACC to ethylene as vegetative plants. It is concluded that flower induction in the short-day plant *Chenopodium rubrum* decreases ethylene production by decreasing their capability of converting ACC to ethylene.

Photoperiodic flower induction in the obligatory short-day plant *Chenopodium rubrum* was shown to be inhibited by application during induction of either auxin (indole-3-acetic acid; IAA) (Krekule and Přívratský 1974, Seidlová and Khatoon 1976) or ethylene in the form of ethephon (Khatoon et al. 1973). Using aminoethoxyvinylglycine (AVG), an inhibitor of ethylene production, and Ag^+ ions, an ethylene antagonist, we have shown that the inhibitory effect

of auxin on photoperiodic flower induction is mediated by ethylene (Macháčková et al. 1985, 1986). A negative correlation between endogenous ethylene production and responsiveness to an inductive cycle of 13 h of darkness was shown in *C. rubrum* plants during the first 3 weeks of growth (Ullmann et al. 1986). All these results point to the possibility of ethylene's playing a role in the regulation of photoperiodic induction of flowering.

To further investigate the role of ethylene in flowering, its endogenous production was followed in vegetative and to flowering induced *C. rubrum* plants. After finding significant differences, we have further investigated which step in ethylene synthesis photoperiodic flower induction influences. Conjugation of ACC to MACC was also examined.

Materials and Methods

Plant Cultivation

Chenopodium rubrum L. (ecotype 374) plants were germinated and cultivated as described by Ullmann et al. (1985). The seedlings were grown in half-strength Knop's solution under continuous illumination (8000 1 ×) at 20 ± 1°C up to an age of 5 days. Control plants were grown further under continuous illumination, and the other two plant batches were submitted to photoperiodic induction by three cycles of 12 h darkness and 12 h light. In one batch the inductive floral effect was canceled by a night break of 2 h light in the middle of each dark period. Twelve hours after the end of the last dark period, the seedlings were analyzed for ethylene production, endogenous ACC and MACC content, and ACC conversion to ethylene. Flowering was scored in samples of all three treatments 1 week after the end of induction.

Ethylene Determination

Ethylene production was followed in sealed Plexiglas boxes (volume 35 ml) stoppered with a rubber septum. Fifty plants were incubated for 2 h in light at 20°C. At the end of incubation, two samples of atmosphere were withdrawn using hypodermic syringes and immediately analyzed by gas chromatography.

ACC and MACC Analysis

Plants were cut into shoots and roots and immediately frozen in solid CO_2. Frozen material was homogenized in 80% ethanol and extracted for 20 h at 4°C. After filtration the extracts were evaporated to the water phase, and this was frozen and thawed to remove chlorophyll. The extracts were then divided into two halves and evaporated to dryness. One half was used for ACC analysis; the other was hydrolyzed by 2 ml 2 N HCl for 5 h at 110°C. The acidic hydrolyzates were neutralized, and the samples were evaporated to dryness and used for ACC + MACC analysis. The ACC content was determined ac-

cording to Lizada and Yang (1979) as modified by C. Bergner (private communication). NaOBr was used as an oxidant.

Conversion of ACC at Saturating Concentration to Ethylene

Five-day-old *C. rubrum* plants were further grown under continuous light or submitted to three cycles of 12 h dark, 12 h light. Analyses were made 12 h after the end of the last dark period. Plants were preincubated (only roots in the solution) for 1 h in a solution of 5 mol · m^{-3} ACC (which is a saturating concentration for these plants) at 20°C in light and further incubated in a closed vessel for 2 h under the same conditions. Evolved ethylene was analyzed by gas chromatography.

Conversion of Labeled ACC to Ethylene and MACC

[2,3-^{14}C]ACC was synthesized according to Schöllkopf et al. (1973) by adding [U-^{14}C]dibromoethane to ethylisocyanoacetate. The specific activity of the ^{14}C-ACC obtained was 61 MBq · $mmol^{-1}$.

One hundred plants placed on a nylon net support were transferred to a box (volume 90 ml) with 6 ml of a ^{14}C-ACC solution (final concentration 50 nmol · ml^{-1}, final activity 5.10^5 Bq · ml^{-1}) so that only roots were in contact with the solution. Plants were preincubated in this solution for 2 h at 20°C in light. Then the box was sealed and connected to a flow-through system designed after Tykva (1964). The evolved labeled ethylene was trapped in an absorbing vessel with 2 ml of saturated solution of mercuric acetate in methanol cooled from the outside by ethanol with solid CO_2. Incubation lasted 3 h (20°C, light). At the end of the incubation, the mercuric acetate solution was transferred to a scintillation vial, and after addition of 6 ml of scintillation solution, radioactivity was counted with a Packard scintillation counter. Plants were washed and immediately frozen. They were homogenized in 80% ethanol. ACC and MACC in the extracts were separated by ion-exchange chromatography (Lizada and Yang 1979), and their content was determined by counting the radioactivity of respective eluates from the ion-exchange columns.

All results are the means of two independent experiments, in which all analyses were performed twice.

Results

Induced *C. rubrum* plants (100% flowering) produced 42% less ethylene than the vegetative ones (Table 1). Night-break canceled flowering and raised ethylene production almost to the level of vegetative plants. However, the levels of ACC were comparable in shoots and roots of all treatments, the mean amounting to about 2 nmol · g^{-1} dry weight (Table 2).

The MACC level in the shoots of *C. rubrum* was also low, about 0.6 nmol · g^{-1} dry weight and was not much different among the treatments (Table 3).

Table 1. Ethylene production in intact 8-day-old *C. rubrum* plants kept under continuous light (vegetative control), induced to flower by three cycles of 12 h darkness, 12 h light at the age of 5 d or given a night-break (each dark period interrupted by 2 h light in the middle).

Treatment	Ethylene production ($nl \cdot g^{-1}$ dry weight $h^{-1} \pm$ SE)	Flowering (%)
Continuous light	63.2 ± 2.1	0
Three cycles (12 h dark, 12 h light)	36.7 ± 1.5	100
Night-break	59.1 ± 2.8	0

Table 2. ACC content in the shoots and roots of 8-day-old *C. rubrum* plants kept under continuous light (vegetative control), induced to flower by three cycles of 12 h darkness, 12 h light at the age of 5 d or given a night-break (each dark period interrupted by 2 h light in the middle).

Treatment	Plant part	ACC ($nmol \cdot g^{-1}$ dry weight ± SE)	Flowering (%)
Continuous light	Shoots	1.8 ± 0.1	0
	Roots	2.1 ± 0.2	
Three cycles (12 h dark, 12 h light)	Shoots	1.7 ± 0.1	100
	Roots	2.1 ± 0.3	
Night-break	Shoots	1.7 ± 0.1	0
	Roots	2.0 ± 0.1	

Surprisingly, much higher levels of MACC were found in roots, especially when plants were given three dark cycles (Table 3).

It is to be noted that the capability to convert the saturating concentration of ACC ($5\ mol \cdot m^{-3}$) to ethylene in the induced plants was only 30% of that in the vegetative plants (Table 4). The investigation with 2,3-[^{14}C]ACC revealed that there was no difference in ACC uptake among the three treatments. In all treatments about 33% of the applied ACC was taken up, 81% of this amount remained unchanged (Table 5), and about 11% was converted to MACC. Conversion of ACC to ethylene was rather low (1.5–2.4%). However, this reaction was influenced by flower induction: induced plants converted 1.5% of the ACC taken up to ethylene, whereas vegetative plants converted 2.4%, a difference of over 50%. Plants subjected to night-break converted 2.2%, almost as much as vegetative ones (Table 5).

Discussion

Decreased ethylene production was observed in plants of *C. rubrum* induced to flower. This is in accordance with the previous observations that endogenous ethylene production was negatively correlated with the capacity for flowering (Ullmann et al. 1986) and that applied ethylene (ethephon) exerted inhibitory effect on flower induction (Khatoon et al. 1973). However, this result is

Table 3. MACC content in the shoots and roots of 8-day-old *C. rubrum* plants kept under continuous light (vegetative control), induced to flower by three cycles 12 h darkness, 12 h light at the age of 5 d or given a night-break (each dark period interrupted by 2 h light in the middle).

Treatment	Plant part	MACC (nmol · g^{-1} dry weight ± SE)	Flowering (%)
Continuous light	Shoots	0.5 ± 0.1	0
	Roots	21.4 ± 0.8	
Three cycles (12 h dark, 12 h light)	Shoots	0.6 ± 0.1	100
	Roots	88.3 ± 2.3	
Night-break	Shoots	0.6 ± 0.1	0
	Roots	82.0 ± 3.0	

Table 4. Conversion of saturating ACC concentration (5 mM) to ethylene by 8-day-old *C. rubrum* plants kept under continuous light (vegetative control), induced to flower by three cycles of 12 h darkness, 12 h light at the age of 5 d or given a night-break (each dark period interrupted by 2 h light in the middle).

Treatment	Ethylene production (nl · g^{-1} dry weight · h^{-1} ± SE)	Flowering (%)
Continuous light	803 ± 41	0
Three cycles (12 h dark, 12 h light)	246 ± 23	100
Night-break	715 ± 46	0

opposite to that reported recently for spinach (Crevecoeur et al. 1986). Spinach plants induced to flower were reported to produce more ethylene than the vegetative ones. There may be different ethylene action in a long-day (such as spinach) and a short-day (such as *C. rubrum*) plant. The changes in ethylene production in spinach were considered to be a response rather to the changed light regime, which need not directly be connected with flowering. The night-break experiments with *C. rubrum* showed that in this plant decreased ethylene production is under the same photoperiodic control as flowering, and thus these two phenomena might be related.

The above results raise the question of which step in ethylene synthesis—ACC synthesis (or its conjugation to MACC) and/or its conversion to ethylene—was correlated to flower induction. Our results show that ACC levels were the same in plants of all three experimental treatments and thus were not interrelated to flower induction. The ACC levels were rather low, which may be accounted for by the fact that the experiments were carried out with young growing plants showing a low ethylene production (Ullmann et al. 1986). Also the MACC level was found not to be under the influence of flower induction. Its level was rather low in all cases with the exception of roots, especially in treatments with three dark cycles. Night-break treatment shows that this increase is unlikely to be connected with flowering. The cause of elevated MACC level in roots is unknown.

Table 5. Conversion of [^{14}C]ACC to MACC and ethylene in 8-day-old *C. rubrum* plants kept under continuous light (vegetative control), induced to flower by three cycles of 12 h darkness, 12 h light at the age of 5 d or given a night-break (each dark period interrupted by 2 h light in the middle).[a]

Treatment	ACC (% radioactive ACC taken up)	MACC	Ethylene	Flowering (%)
Continuous light	83.6 ± 4.3	8.5 ± 0.4	2.4 ± 0.3	0
Three cycles (12 h dark, 12 h light)	80.1 ± 2.7	9.5 ± 0.9	1.5 ± 0.2	100
Night break	80.4 ± 2.4	10.3 ± 1.2	2.2 ± 0.2	0

[a] The percentages of ACC taken up by these three treatments were 30.9 ± 3.6%, 32.9 ± 4.1%, and 32.3 ± 3.8%, respectively.

Detailed study of [^{14}C]ACC metabolism showed clearly that the only reaction affected by the inductive photoperiodic treatment is the conversion of ACC to ethylene. It was suppressed in induced plants by more than 30%. The low conversion of ACC taken up to ethylene can probably be explained, as can the low level of endogenous ACC, by the material used being young growing plants with low ethylene production.

The effect of the inductive photoperiodic treatment on ACC conversion to ethylene was confirmed in experiments with ACC at saturating concentration. This reaction is considered to be a measure of ethylene-forming enzyme (EFE) activity in plants (Kao and Yang 1982).

We may thus conclude that photoperiodic flower induction in the short-day plant *Chenopodium rubrum* brings about reduction of ethylene production via decreased ACC conversion to ethylene. The significance of this finding should be further investigated in terms of its specificity in flower induction.

Our present results support the view that the high level of ethylene is detrimental to flowering in short-day plants of *C. rubrum* (Khatoon et al. 1973, Ullmann et al. 1986). However, this conclusion was based on experiments using applied ethylene at concentrations much greater than those under natural conditions. Further information, especially on the exact time course of ethylene production during flower induction, is needed to clarify the question.

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