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Ethylene Production and Metabolism of 1-Aminocyclopropane-1-Carboxylic Acid in a Long-Day Plant, *Chenopodium murale* L., as Influenced by Photoperiodic Flower Induction

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Abstract. *Chenopodium murale* plants, induced to flower by 5 days of continuous light, produced 43% more ethylene than vegetative plants kept under short days (16 h darkness, 8 h light). The 1-aminocyclopropane-1-carboxylic acid (ACC)-induced ethylene production, using saturating ACC concentration (10 mol · m^{-3}) was also 55% higher in induced plants. Their ACC and N-malonyl-ACC (MACC) levels were also higher, the former increasing by 56% in both shoots and roots, the latter by 288% and 108% in shoots and roots, respectively. Administration of labeled [2,3-^{14}C]ACC produced a very similar relative content of ACC and MACC in both treatments. The only process influenced by flower induction was ACC conversion to ethylene. Induced plants converted 66% more ACC than the vegetative ones. The effects of photoperiod on ethylene formation and metabolism in a long-day plant (LDP) *C. murale* and a short-day plant (SDP) *C. rubrum* are compared. Ethylene formation seems to be under photoperiodic control in both species, but its role in flower induction remains obscure.

Photoperiodic flower induction in the obligatory SDP *Chenopodium rubrum* was recently shown to be accompanied by a decrease in ethylene formation (Macháčková et al. 1988). The contents of ACC and MACC did not change during the induction. Experiments using labeled ACC established that the reaction influenced by flower induction manifested itself as ACC to ethylene

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; MACC, N-1-malonylaminocyclopropane-1-carboxylic acid; SDP, short-day plant; LDP, long-day plant.

conversion, while on the other hand, night-break experiments proved this reaction to be under photoperiodic control.

In 1986 Crevecoeur et al. performed a similar study with LDP *Spinacia oleracea*. They found the opposite situation to that in *C. rubrum*—i.e., increased ethylene production by whole plants and isolated leaves induced to flowering by 1 day of continuous illumination. Also, ACC-induced ethylene production was increased, and the ACC content was decreased in induced plants.

We studied ethylene production and metabolism in induced and vegetative plants of a species closely related to *C. rubrum*, an LDP *Chenopodium murale* L., to determine the degree of involvement of the photoperiodic control of ACC to ethylene conversion, its potential significance to flower induction, and the difference between SDPs and LDPs.

Materials and Methods

Plant Cultivation

Chenopodium murale L. (ecotype 197) plants were germinated and cultivated as described by Macháčková et al. (1986) with some modifications. The seeds were germinated for a minimum of 2 days under alternating light (12 h) and darkness (12 h), the temperature being 30 ± 1°C during the light and 5 ± 1°C during the darkness. The seedlings were grown in perlite, watered daily with half-strength Knop's nutrient solution at 20 ± 1°C under short days (16 h darkness, 8 h light, fluorescent tubes, 8000 lx), up to an age of 23 days. Control plants were grown under short days; some plants were exposed to photoperiodic induction by 5 days of continuous illumination using incandescent lamps (8000 lx). The plants were analyzed for ethylene production, their endogenous ACC and MACC levels, and ACC to ethylene conversion rate 4 h after the end of the dark period in control plants and at the same time of the day in induced plants.

Flowering was scored in samples of both treatments 1 week after the end of induction using a stereomicroscope.

Ethylene Determination

Ethylene production was determined as described by Macháčková et al. (1988), but with only 15 plants being used for one determination.

ACC and MACC Analysis

ACC and MACC were extracted with 80% ethanol. After evaporation (chlorophyll being removed by freezing and thawing), and after hydrolysis by 2 N HCl in the case of MACC, the ACC content was determined according to Lizada and Yang (1979) in the modification with NaOBr as an oxidant (Macháčková et al. 1988).

Table 1. Endogenous and ACC-induced ethylene production in intact 28-day-old *C. murale* plants kept under short days (16 h darkness, 8 h light—vegetative control) or induced to flower by 5 days of continuous illumination. ACC saturating concentration was used (10 mol · m^{-3}).

Light regimen	Ethylene production (nl · $g^{-1}d$ · wt h^{-1} ± SE)	Flowering (%)
Short days		
−ACC	115.3 ± 12.8	0
+ACC	937.6 ± 56.3	
5 days continuous light		
−ACC	164.7 ± 15.1	80
+ACC	1453.3 ± 82.7	

Conversion of ACC at Saturating Concentration to Ethylene

Twenty-eight-day-old *C. murale* plants grown under SD or exposed to 5 days of continuous illumination were preincubated for 1 h (only roots in the solution) in a solution of 10 mol · m^{-3} ACC at 20 ± 1°C in light and further incubated in a closed vessel (15 plants, 35 ml) for 2 h under the same conditions. The 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). Specific activity of the preparation was 61 MBq · $mmol^{-1}$. Incubation and measurements were performed as described by Macháčková et al. (1988), but only 50 plants were used for the analysis.

All results are the mean of two separate experiments during which all analyses were performed twice.

Results

Induced plants of *C. murale* (80% flowering) produced 43% more ethylene than the vegetative ones (Table 1). ACC-induced ethylene production using saturating ACC concentration (10 mol · m^{-3}) was also higher in induced plants (55%) (Table 1).

The ACC and MACC levels increased in both the shoots and roots of the induced plants. The content of ACC was comparable in shoots and roots and increased therein upon induction by 56% (Table 2); that of MACC was higher in the roots of the plants exposed to both treatments. Its content in the shoots and roots of induced plants was 288% and 108% higher, respectively (Table 2).

The experiments using [2,3-^{14}C]ACC revealed that the two treatments produced no difference in ACC uptake. In both about 35% of the applied ACC was taken up, of which 80% remained unchanged and about 10% was converted to MACC (Table 3). Conversion of ACC to ethylene was rather low (1.2–2.0%). However, this reaction was influenced by induction: induced plants converted

Table 2. ACC and MACC content in the shoots and roots of 28-day-old *C. murale* plants kept under short days (16 h darkness, 8 h light—vegetative control) and induced to flower by 5 days of continuous illumination.

Light regimen	Plant part	ACC (nmol · g^{-1}d · wt ± SE)	MACC (nmol · g^{-1}d · wt ± SE)	Flowering (%)
Short days	Shoots	3.0 ± 0.2	9.8 ± 0.8	0
	Roots	3.2 ± 0.3	49.5 ± 3.2	
5 days continuous light	Shoots	4.7 ± 0.4	38.0 ± 3.4	80
	Roots	5.0 ± 0.4	102.7 ± 10.9	

Table 3. Conversion of [2,3-^{14}C]ACC to MACC and ethylene in 28-day-old *C. murale* plants kept under short days (16 h darkness, 8 h light—vegetative control) and induced to flower by 5 days of continuous illumination. Percentages of ACC taken up by plants in both treatments were 33.2 ± 3.4 and 36.5 ± 3.7%, respectively. Data given are expressed in % of taken up radioactive ACC.

Light regimen	Nonconverted ACC (% of radioactive ACC taken up ± SE)	MACC (% of radioactive ACC taken up ± SE)	Ethylene (% of radioactive ACC taken up ± SE)	Flowering (%)
Short days	82.1 ± 7.6	9.7 ± 1.1	1.2 ± 0.1	0
5 days continuous light	78.6 ± 8.1	10.6 ± 1.3	2.0 ± 0.2	80

2.0% of the ACC taken up to ethylene, whereas vegetative plants converted 1.2%, resulting in a difference of 66% (Table 3).

Discussion

Similarly to spinach (Crevecoeur et al. 1986), *Chenopodium murale* showed increased ethylene formation by whole plants shortly after the end of photoperiodic flower induction. The same was true for ACC-dependent ethylene formation. Saturating ACC concentration was used in *C. murale*, and the obtained value can thus be considered to represent the EFE (ethylene-forming enzyme) activity (Kao and Yang 1982). This conclusion was substantiated by experiments with labeled ACC showing that its conversion to ethylene responded to inductive treatment. Unlike in spinach, where the ACC level decreased, in *C. murale* plants, flower induction led to a rise in levels of ACC and MACC. The difference might be due to longer treatment of *C. murale* with continuous incandescent light. The marked increase of MACC content in the induced plants, especially in their shoots, might be ascribed to the promotive effect of light on ACC malonylation (Jiao et al. 1987).

Comparison of induced SD *C. rubrum* (Macháčková et al. 1988) and LD *C. murale* plants with respect to ethylene production reveals their opposite character. Induced plants of *C. murale* produce more ethylene than the vegetative ones; those of *C. rubrum* produced less. Both species responded to light reg-

imen by changing the rate of ACC to ethylene conversion; yet the increased ACC level in *C. murale* cannot be neglected. This comparison strongly suggests that ethylene formation, and particularly the conversion of ACC to ethylene, depends on the light regimen; in both species, the overall ethylene formation and ACC to ethylene conversion were higher under continuous illumination, this light regimen being inductive for *C. murale* and noninductive in *C. rubrum*.

Our results thus lead to a similar conclusion as that of Crevecoeur et al. (1986, 1988)—that the changes in ethylene release and ACC to ethylene conversion rate appear to manifest a more direct response to light regimen than to inductive conditions of flowering. It seems that changes in ethylene production might characterize the "induced state," at least to a certain degree. The assessment of this degree as well as of the role of ethylene in photoperiodic flower induction requires further thorough studies.

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