J Plant Growth Regul (1987) 6:201–208

Journal of
Plant
Growth
Regulation

Chilling-Induced Ethylene Production by Beans and Peas

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Received March 16, 1987; accepted July 14, 1987

Abstract. The effects of chilling on ethylene production by leaf discs and whole plants of bean (chilling-sensitive) and pea (chilling-tolerant) were studied. When pea or bean leaf discs were excised and incubated at 25°C, transient increases in ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) accumulation were observed. Both pea and bean discs kept at 5°C evolved little ethylene, but levels of ACC increased in pea discs and not in bean discs. When discs of either species were chilled at 5°C immediately after excision and then transferred to 25°C 9 h later, increases in their ACC levels and ethylene production rates were observed. Discs were also incubated at 25°C for 12 h to allow excision-induced ethylene production to subside and then chilled at 5°C. Nine hours later, these discs were transferred to 25°C, and an increase in ethylene production was observed. These data indicate that chilling suppresses excision-induced ethylene production and enhances the production of ethylene after transfer to 25°C. Chilling of whole plants resulted in increased production of ethylene and ACC in the chilling-sensitive bean but not in the chilling-tolerant pea. Treatment of bean plants with the ethylene antagonists silver thiosulfate, norbornadiene, or aminooxyacetic acid, or of pea plants with ethylene, did not affect the appearance of chilling injury symptoms, indicating that ethylene does not induce injury symptoms and may not have an adaptive role in chilling stress.

The production rate of ethylene by plant tissues is normally low but is stimulated when the tissues are stressed. Chilling treatments have been shown to stimulate ethylene production by various plants upon their transfer to warmer temperatures (Chan et al. 1985, Chen and Patterson 1985, Cooper et al. 1969, Field 1981, Wang and Adams 1980). It has therefore been suggested that ethylene production may be used as an indicator of chilling sensitivity (Chen and Patterson 1985).

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The increased ethylene production induced by a chilling treatment has been shown to result from stimulated synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene. Levels of ACC increase in chilled cucumber fruit (Wang and Adams 1982) or dwarf bean leaf discs (Field 1984), both chilling-sensitive species, only after transfer to a warmer temperature. However, ACC levels increase in *Episcia reptans*, also a chilling-sensitive species, at 0°C (Chen and Patterson 1985). In this study, we examined the effect of chilling on ethylene and ACC production by leaf discs and whole plants of beans (chilling-sensitive) and peas (chilling-tolerant).

Materials and Methods

Plant Materials

Prior to planting, bean (*Phaseolus vulgaris* L. cv. Blue Lake Bush) seeds were imbibed on paper towels and soaked with 0.2 M $CaCl_2$ for 2 h. Pea (*Pisum sativus* L. cv. Alaska) seeds were imbibed in aerated water for 6 h. The seeds were then planted in vermiculite and grown in a growth chamber under a 16-h photoperiod (540 μE PAR m^{-2} s^{-1}) at 25°C day/20°C night at 60% relative humidity. Ten to 14 days after planting, experiments were initiated using whole plants or 7-mm-diameter leaf discs.

Chilling Treatments

Discs were cut at 25°C and immediately placed into 5-ml test tubes, with each tube containing 10 discs. The tubes were then transferred to controlled temperature rooms held at 5°C or 25°C and placed in beakers partially filled with water previously equilibrated at 5°C or 25°C. To determine whether chilling affected ethylene production after the excision-induced ethylene production had subsided, leaf discs were cut, floated on water for 12 h in darkness at 25°C, and then transferred to test tubes and treated as above. Discs were chilled at 5°C for 9 h, after which half the samples were transferred to 25°C. Temperature treatments were conducted in darkness. Whole plants were chilled at 5°C either in darkness, in 66-L containers continually flushed with air at 4 L h^{-1}; or in light, on a bench under a bank of fluorescent lights. The plants were warmed by transferring them into 1-L glass jars (1 plant per jar) held at 25°C. The jars were covered with aluminum foil so that the plants would be kept in darkness.

Inhibitor, ACC, and Ethylene Treatments

To determine the capability of whole plants to produce ethylene from ACC after being chilled for 1 or 10 days, the plants were transferred from 5°C to 25°C. After equilibrating at 25°C for 1 h, the plants were sprayed until runoff with a solution containing 1 mM ACC and 0.1% (v/v) Tween 20 and then enclosed in 1-L jars. After 1 h, 1-ml air samples were taken from the air spaces of

the jars for ethylene measurements. To determine if ethylene affected the appearance of chilling injury symptoms, whole plants were treated with either 3 $\mu l\ L^{-1}$ ethylene, 250 $\mu l\ L^{-1}$ norbornadiene, 100 μM aminooxyacetic acid (AOA), or 2 mM silver thiosulfate just before, during, or after chilling at 5°C in darkness. After being chilled for 10 days, the plants were transferred to 25°C in light and scored for chilling injury symptoms (leaf necrosis, wilting, death). Control plants were kept in air or treated with sodium thiosulfate.

Ethylene and ACC Determinations

Ethylene production was measured from plant material kept in static systems. Leaf discs were enclosed in test tubes for 1 h, after which ethylene was sampled from the air space of the tubes. The tubes were then flushed with air and resealed for the next measurement. Whole plants were enclosed in 1-L jars covered with aluminum foil. One hour after enclosure, air samples were removed from the air spaces of the jars for ethylene measurement, after which leaf tissues (1 of a pair of primary leaves of bean or 0.25 g fully expanded pea leaves) were taken for ACC determinations.

Ethylene was measured using a gas chromatograph equipped with an alumina column and a flame ionization detector. For ACC determinations, leaf tissue was extracted in 80% (v/v) ethanol at 60°C until the tissue was no longer green. The ethanol was then evaporated to dryness, and the residue was dissolved in 0.5 ml chloroform and 2.5 ml water and thoroughly mixed with a vortex mixer. An aliquot of the aqueous extract was taken for the ACC assay, according to the method of Lizada and Yang (1979).

All experiments were done at least twice, using two or three replicates of each sample.

Results and Discussion

Ethylene Production from Leaf Discs

Excision-induced ethylene production was detectable during incubation at 25°C from both pea and bean leaf discs. When the discs were chilled at 5°C immediately after being excised, the increase in ethylene production from the discs was not detected. When the discs were transferred to 25°C after being chilled at 5°C for 9 h, an increase in ethylene production occurred over that at 5°C (Figs. 1A, 2A).

The data in Fig. 1A corroborate those of Field (1981), who found that ethylene production from dwarf bean leaf discs at 5°C is low but "overshoots" after transfer to 25°C. This "burst" of ethylene production by leaf discs after transfer to 25°C, according to Field, may be due to chilling-induced enhancement of excision-related ethylene production. By adding exogenous ACC to leaf discs at 5°C, Field (1984) further showed that the conversion of ACC to ethylene is not completely inhibited, and thus that synthesis of ACC is the limiting factor at 5°C. Measurements of ACC content in bean leaf discs after

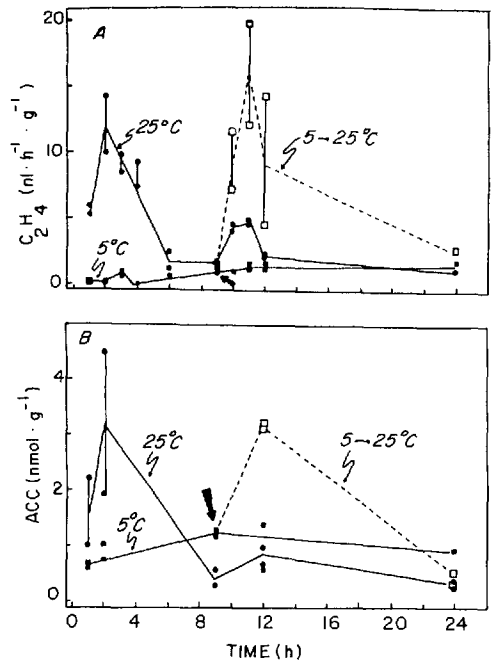

Fig. 1. Time course of ethylene production (A) and ACC accumulation (B) by bean leaf discs after excision. Discs were cut at 25°C and then incubated at 25°C (solid circles) or 5°C (solid squares). Half the samples incubated at 5°C were transferred to 25°C after 9 h (open squares), as indicated by the arrow. Vertical lines indicate the range between two replicates. Single points indicate that the replicate values were the same.

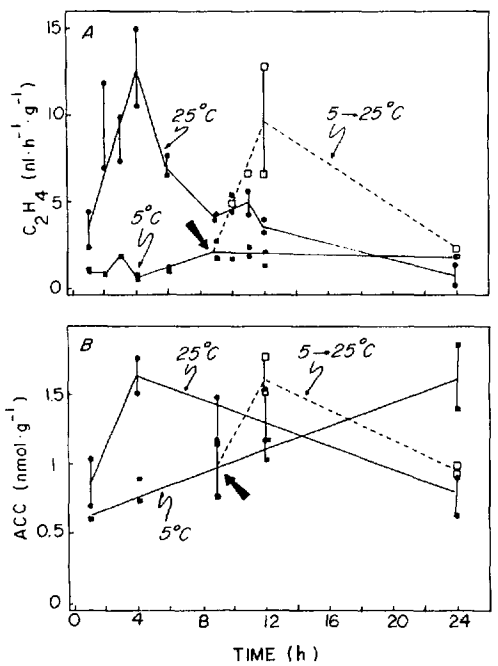

Fig. 2. Time course of ethylene production (A) and ACC accumulation (B) by pea leaf discs after excision. Treatments were as in Fig. 1.

transfer from 5°C to 25°C (Field 1984) suggest that ACC does not accumulate at low temperature but does so only upon transfer to warmer temperatures.

As shown in Fig. 1B, levels of ACC in bean leaf discs kept at 5°C remained low and constant over time. Upon transfer from 5°C to 25°C, levels of ACC in the bean leaf discs increased after transfer and then decreased, in parallel with ethylene production. These data indicate that chilling delays the excision-enhanced increase in ethylene production by delaying the synthesis of ACC. Although the variation in the 2-h measurements of ACC for discs incubated at 25°C was high in Fig. 1B, five repetitions of this experiment indicated that ACC accumulation consistently paralleled ethylene production at 25°C. In contrast to the bean discs, ACC levels in pea leaf discs increased during incubation at 5°C (Fig. 2B). Although the increase in ACC was only two- to threefold, separate experiments have shown (data not presented) that it can increase as much as sixfold. After transfer from 5°C to 25°C, ACC levels increased slightly and then decreased. Accumulation of ACC in both bean and pea leaf discs kept at 25°C paralleled synthesis of ethylene.

To separate effects of chilling from those of excision on ethylene production, excision-induced ethylene was allowed to subside before the leaf discs were chilled. Figure 3 shows that bean and pea leaf discs treated in this manner, then incubated at 5°C or 25°C, produced low levels of ethylene 21–24 h after excision. Transfer of the discs at 5°C to 25°C resulted in a stimulation of ethylene production comparable to that shown in Figs. 1A and 2A. These data indicate that chilling enhances the production of ethylene after transfer from 5°C to 25°C.

Effects of Chilling on Whole Plants

When whole bean plants were chilled in light, they developed leaf necrosis and inrolling after only 1 day. If chilled in darkness, these symptoms of chilling injury required more time to develop (3–6 days) and were observed only after transfer from 5°C (dark) to 25°C (dark or light). After 14 days of continuous chilling in darkness, fungi grew on the leaves of the bean plants, but no necrosis or inrolling was observed while the plants were kept at 5°C. Pea plants exhibited no visible symptoms of chilling injury even after 10 days of chilling in darkness or in light, even if transferred to 25°C.

Ethylene production by bean plants at 5°C in darkness increased with length of the chilling treatment (Fig. 4A). ACC levels in the leaf tissues of these plants increased as the ethylene production increased (Fig. 4B). Bean plants transferred from 5°C to 25°C produced more ethylene and contained higher ACC levels than corresponding plants at 5°C. This increase in ACC levels at 25°C after transfer from 5°C in darkness occurred under either light or dark conditions and started 1 h after transfer (Fig. 5). In contrast, the ethylene production rate and ACC content of pea plants remained low and constant at 5°C or after transfer from 5°C to 25°C (Fig. 4).

To determine if the capability of the plants to convert ACC to ethylene changed over time because of chilling, the plants were transferred to jars at 25°C after 1 or 10 days of chilling in darkness at 5°C and sprayed with a solu-

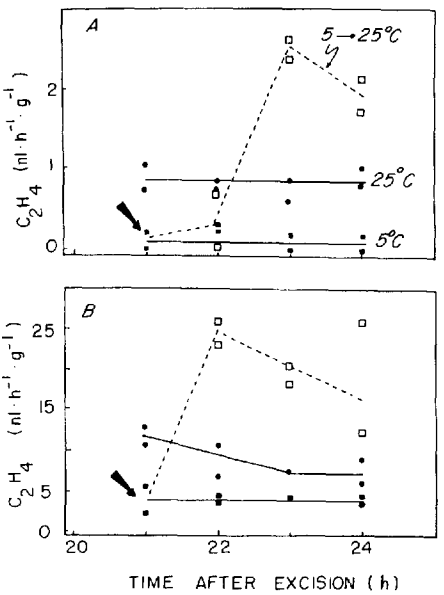

Fig. 3. Time course of chilling-induced ethylene production by bean (A) and pea (B) leaf discs. Discs were cut and floated on water in the dark for 12 h at 25°C to let the excision-induced ethylene production subside, and then they were transferred to test tubes and incubated at 25°C (solid circles) or 5°C (solid squares). Half the samples incubated at 5°C for 9 h were subsequently transferred to 25°C (open squares).

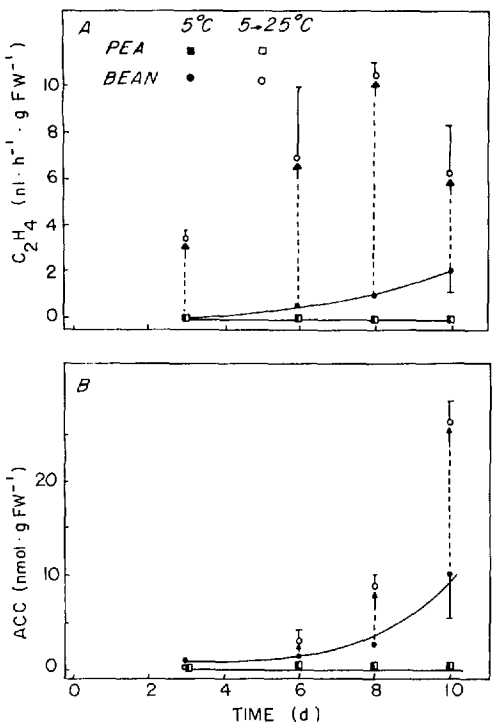

Fig. 4. Ethylene production (A) and ACC accumulation (B) by whole bean (circles) and pea (squares) plants. Plants were chilled at 5°C in closed containers under continual airflow. After 3, 6, or 10 days at 5°C, each plant was transferred to a 1-L jar either at 5°C (solid symbols) or 25°C (open symbols) in darkness, and the jars were sealed for ethylene determination between 1 and 2 h after transfer. The arrows designate the increase measured in ethylene or ACC production after the bean plants were transferred from 5°C to 25°C. After ethylene was assayed, leaf tissue was removed from the plants and extracted for ACC as described in Materials and Methods. Vertical lines indicate standard error of the mean of three replicates.

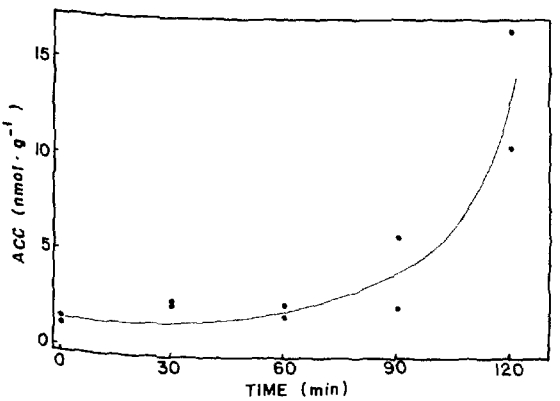

Fig. 5. Time course of ACC accumulation in leaves of whole bean plants chilled for 10 days in darkness at 5°C and then transferred to 25°C in the light. At 0, 30, 60, 90, and 120 min after the plants were transferred to 25°C, one of a pair of primary leaves was excised, weighed, and then assayed for ACC content. Two different halves of leaf pairs were used for each data point.

tion containing 1 mM ACC and 0.1% Tween 20. After 1 h, the jars containing the plants were sealed, and ethylene was sampled from the air space of the jars 1 h later. There was little change in the capability to convert ACC to ethylene with chilling.

The data shown in Fig. 4 indicate that, as Chen and Patterson (1985) have suggested, ethylene production and ACC accumulation by whole plants during chilling treatment may be indicators of chilling sensitivity. To substantiate this hypothesis, a more extensive survey of chilling-sensitive and chilling-tolerant species must be conducted. A comparison of data obtained with leaf discs and with whole plants indicates that leaf discs cannot be used as systems for estimating chilling sensitivity of the whole plant, because the accumulation of ACC occurred in bean leaves when the whole plant was chilled but not when the discs were chilled (compare Figs. 1 and 4).

To determine whether ethylene has a physiological role in the development of chilling injury symptoms, bean plants were treated with 250 $\mu l\ L^{-1}$ norbornadiene, 2 mM silver thiosulfate, or 100 μM AOA before, during, or after chilling. Norbornadiene and Ag^+ have been shown to inhibit ethylene action (Beyer 1979, Sisler and Yang 1984, Veen 1983), and AOA inhibits the synthesis of ACC (Yu et al. 1979). Treatment of bean plants with these compounds was expected to alleviate chilling injury symptoms if ethylene caused symptom development. Control bean plants were treated with water or 2 mM sodium thiosulfate. Pea plants were incubated in air or 3 $\mu l\ L^{-1}$ ethylene before, during, or after chilling in the dark to determine whether ethylene could induce symptom development. However, control and treated plants looked the same after 10 days of chilling in the dark followed by transfer to 25°C in the light. Under no treatment was chilling injury alleviated in bean plants or developed in pea plants. These observations indicate that ethylene does not play a role in the development of chilling injury symptoms in bean and pea plants.

Acknowledgments. This work was supported by grant PCM 84-14971 from the National Science Foundation.

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