Method for Overcoming the Antiethylene Effects of Ag⁺¹

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ABSTRACT

A technique is described for eliminating the antiethylene effects of the Ag⁺ ion in the intact pea plant (Pisum sativum). The technique is based on the ability of the ethylene mimic, acetylene, to negate the antiethylene effect of Ag⁺, presumably through salt formation, and subsequently to induce the ethylene response.

The Ag⁺ ion effectively blocks ethylene action. In the initial work with cucumber and tomato (4) a single foliar application of AgNO₃ greatly reduced ethylene sensitivity. Responses to exogenous ethylene counteracted by Ag⁺ included growth retardation, epinasty, senescence, and abscission. Ag⁺ also counteracted endogenous ethylene by shifting sex expression in gynoecious cucumber plants. In similar studies (2, 3), Ag⁺ pretreatment prevented ethylene-induced root growth inhibition and the classical "triple" response in etiolated peas. It also blocked abscission and/or senescence in cotton, bean, and orchids.

Shortly after these reports, Halevy and Kofranek (7) reported that Ag⁺ delayed senescence of cut carnations. Additional work by Veen and van de Geijn (10) has recently suggested an improved method for extending flower longevity using the silverthiosulfate anionic complex rather than AgNO₃. Liu (9) reported using Ag⁺ to counteract the effect of ethylene effectively in senescence spot development of bananas while Beutelmann and Kende (1) used Ag⁺ to inhibit ethylene induced rolling up of rib segments cut from the corolla of Ipomoea tricolor. More recently, Bradford and Dilley (5) applied AgNO₃ to tomatoes to demonstrate convincingly that ethylene is responsible for waterlogging symptoms in these plants.

These studies demonstrate the ability of Ag⁺ to prevent ethylene action. For certain applications it is often difficult to decide whether the Ag⁺ response really represents a specific antiethylene effect. For example, when ethylene promotes a response (e.g. abscission, ripening, seed germination, growth stimulation), the failure to observe the ethylene response following Ag⁺ treatment could be interpreted as either a specific blocking of ethylene action or a general inhibition of metabolism. To distinguish between these two possibilities a technique was developed that would eliminate the Ag⁺ effect and allow the ethylene response to develop, thereby demonstrating the viability of the tissue. The technique is based on the ability of acetylene to negate Ag⁺ action and to induce the ethylene response. Although it is presumed that acetylene negates Ag⁺ action through salt formation, this mechanism has not yet been demonstrated in vivo.

MATERIALS AND METHODS

Pea seeds (Pisum sativum cv. Alaska) were soaked in distilled H₂O for 4 hr, planted in 15-cm pots containing Jiffy-Mix (Jiffy Products Inc., Chicago) combined with sand (3:1, v/v), and watered with distilled H₂O. Seeds were germinated and grown for 3 days in a dark room at a constant 22 C and 55% RH. After binding to 20 uniform seedlings 10 pots were transferred to each of three similar chambers purged with either ethylene-free air (air scrubbed with Purafil, H. E. Burroughs & Assoc., Inc. Chamblee, Ga.), ethylene (0.22 μl/l), or acetylene (240 μl/l). Following a 2-day exposure, five pots from each chamber were sprayed with water plus 0.01% Tween 20 and five pots were sprayed with 20 mg/l of AgNO₃ plus surfactant. Immediately following treatment the seedlings were returned to their respective chambers. All transfers and treatments were made using a green safelight. The following day eight pots (four controls and four AgNO₃-treated pots) were removed from each chamber and two control pots and two AgNO₃-treated pots were placed in two large vacuum containers. One-half of the air was removed from one of the containers (about 380 mm Hg) and atmospheric pressure immediately restored by bleeding back in pure acetylene. After 15 min this vacuum treatment was repeated but atmospheric pressure was restored this time with room air. This procedure of bleeding air back into the chamber was repeated a third time resulting in three consecutive 15-min exposures to first 50% acetylene, and then 25% and 12.5% acetylene. The two pots of control and AgNO₃-treated seedlings in the other vacuum container were subjected to the same reduced pressures but were not exposed to acetylene. Immediately following these vacuum treatments most of the seedlings were returned for an additional 2 days to their respective chambers purged with either air, ethylene (0.22 μl/l), or acetylene (240 μl/l). However, one of the two pots of control and AgNO₃-treated seedlings taken from the ethylene chamber for air or acetylene vacuum treatment was transferred back to the 240 μl/l acetylene chamber for an equal period of 2 days.

RESULTS AND DISCUSSION

Acetylene rapidly reacts with Ag⁺ to form the very insoluble silver acetylide salt. Therefore, exposing Ag⁺-treated plants to acetylene gas is a potential method for eliminating the antiethylene effects of Ag⁺. To test this idea etiolated pea seedlings were first treated with 20 mg/l of AgNO₃ and placed in 0.2 μl/l of ethylene for 2 days. Under these conditions the seedlings failed to respond to ethylene as previously reported (2). In an attempt to overcome this antiethylene effect of Ag⁺ the seedlings were removed from ethylene, treated with 50% acetylene for 30 min, and then returned to ethylene. Although the AgNO₃-treated seedlings clearly showed a greater sensitivity to ethylene when placed back in 0.2 μl/l of the gas, they did not respond fully indicating incomplete removal of Ag⁺. Repeated vacuum treatment during acetylene exposure helped by facilitating the diffusion of acetylene into the tissue but even this treatment still did not completely eliminate the effect of Ag⁺.
Apparently the reaction between Ag⁺ and acetylene, like the reaction between Ag⁺ and —SH groups of the tissue, does not irreversibly sequester Ag⁺ to the point of making it completely ineffective in blocking ethylene action. This is suggested by experiments where the epicotyls of intact pea seedlings, 1 cm in height, were sprayed with 20 or 40 mg/l of AgNO₃ and then allowed to grow an additional 5 cm in 0.22 μl/l of ethylene. The 4 cm of new growth, which was never directly treated with Ag⁺, was also protected from ethylene indicating that sufficient Ag⁺ continued to reach the epicotyl tips to counteract ethylene action. Therefore, even the very low solubility product of silversulfide (8), which would readily form in Ag⁺-treated tissue, does not eliminate Ag⁺ action. Presumably, even at these low treatment concentrations of AgNO₃, sufficient Ag⁺ is still free to act systemically and protect the new growth.

Because of the inability to negate completely the Ag⁺ antiethylene effects, the seedlings, instead of being returned to ethylene after vacuum treatment, were returned to a physiologically equivalent concentration of acetylene. This procedure had the advantage of providing continuous Ag⁺ scavenging as growth proceeded while simultaneously inducing the ethylene response. This modified procedure proved to be very effective.

As evidenced by the similar appearance of the seedlings in Figure 1A and B, 240 μl/l of acetylene essentially duplicated the change in growth habit caused by 0.22 μl/l of ethylene. In this experiment the air control seedlings (not shown) exhibited the very elongated, spindly type of growth characteristic of etiolated seedlings. These controls were over twice as tall as the ethylene-or acetylene-treated seedlings. Based on pea stem elongation tests Burg and Burg (6) reported ethylene to be about 3,000 times more active than acetylene. As indicated by the concentrations used in these experiments with intact seedlings, ethylene was about 1,000 times more active than acetylene. Seedlings treated with 20 mg/l of AgNO₃ after 2 days exposure to ethylene reverted to a more normal growth habit even though they were continuously being exposed to ethylene (Fig. 1C). As indicated above, the Ag⁺ must act systemically because the new growth above the arrow in Figure 1C is protected from ethylene although it was never directly treated. (The 20 mg/l was more effective than before [2] because of the greater height of the seedlings at the time of treatment resulting in a greater total Ag⁺ dose.) In Figure 1D the effectiveness of acetylene in eliminating the antiethylene effect of Ag⁺ can be readily seen. Seedlings treated with Ag⁺ became insensitive to ethylene (Fig. 1C) but when briefly exposed to high concentrations of acetylene and then placed in a physiologically equivalent level of acetylene, they again exhibited a typical ethylene response.

In these experiments the brief exposure of the surfactant controls to high acetylene during vacuum treatment did not cause a noticeable effect. The seedlings treated with Ag⁺ and left continuously in 240 μl/l of acetylene reverted temporarily to a more normal growth habit, and then after one day, they developed typical ethylene symptoms. This indicated that treating plants with Ag⁺ and placing them directly in a physiological concentration of acetylene (e.g. 240 μl/l for peas), without a brief vacuum treatment to high acetylene, will also overcome the Ag⁺ effect. However, a longer exposure is needed than when the vacuum treatment is included. This was found to be true in recent unpublished experiments where cotton, cucumbers, and tomatoes were sprayed with antiethylene levels of AgNO₃ and then treated continuously for 1 week with either ethylene or a physiological equivalent amount of acetylene. In acetylene, the effectiveness of Ag⁺ diminished much more rapidly than in ethylene. However, it was important to apply only that amount of AgNO₃ needed just to override the ethylene response. Excessive amounts of Ag⁺ tended to prolong the period of acetylene exposure required to negate the Ag⁺ effect.

The treatments reported here clearly demonstrate the ability of acetylene to reverse the antiethylene effect of Ag⁺. Because of the simplicity of the method and the straightforwardness of the results, it should be a useful technique for verifying the viability of tissues treated with Ag⁺. This is especially critical where the antiethylene response produced by Ag⁺ treatment is inhibition.

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**LITERATURE CITED**

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