Deferral of Senescence and Abscission by Chemical Inhibition of Ethylene Synthesis and Action in Bean Explants

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ABSTRACT

Three compounds known to inhibit ethylene synthesis and/or action were compared for their ability to delay senescence and abscission of bean explants (Phaseolus vulgaris L. cv Contender). Aminoethoxyvinylglycine (AVG), AgNO₃, and sodium benzoate were infiltrated into the petiole explants. Their effect on abscission was monitored by measuring the force required to break the abscission zone, and their effect on senescence was followed by measuring chlorophyll and soluble protein in the distal (pulvinus) sections. AVG at concentrations between 1 and 100 micromolar inhibited ethylene synthesis by about 80 to 90% compared to the control during sampling periods of 24 and 48 hours after treatment. This compound also delayed the development of abscission and senescence. Treatment with AgNO₃ at concentrations between 1 and 100 micromolar progressively reduced ethylene production, but to a lesser extent than AVG. The effects of AgNO₃ on senescence and abscission were quite similar to those of AVG. Sodium benzoate at 50 micromolar to 5 millimolar did not inhibit ethylene synthesis during the first 24 hours, but appreciably inhibited ethylene synthesis 48 hours after treatment. It also delayed the development of abscission and senescence. The effects of AVG, Ag⁺, and sodium benzoate suggest that ethylene could play a major role in both the senescence induction phase and the separation phase in bean explants.

Ethylene has been recognized to be a potent promoter of both senescence and abscission of plant parts for many decades. Ethylene appears to lower auxin activity in the abscission zone by changing its synthesis, transport, destruction, or conjugation, and makes the abscission zone more susceptible to separation events (13, 19). The pulvinar tissue of several species produces high levels of ethylene during senescence and prior to separation (16, 24). In intact cotton cotyledons, both ethylene production rates and internal levels are known to reach physiologically active levels prior to senescence (13). The overall role of ethylene in the abscission process has been well established since the early 1970s (16, 20, 23, 24).

Owens et al. (21) observed that the rhizobitoxine analog AVG Effectively inhibited ethylene synthesis in apple and banana fruit discs and pea seedlings. Several ethylene responses such as flower abscission, fruit ripening, Chl breakdown, and loss of membrane permeability were inhibited in the presence of AVG (1–3, 8, 17, 26, 29–31). Compounds known to stimulate ethylene synthesis were not effective in the presence of AVG (18). AVG has been shown to be an irreversible inhibitor of pyridoxal phosphate-dependent enzymes (25) and a powerful inhibitor of ACC synthase (14, 31), the enzyme involved in the conversion of SAM to ACC. Sagee et al. (27) recently found that AVG reduced ethylene production and delayed abscission of citrus leaf explants.

Silver ions were also observed to inhibit ethylene action and metabolism (9, 11, 12, 28). Beyer (10) showed that Ag⁺ counteracted the effect of exogenously applied ethylene on leaf epinasty, abscission, and senescence of tomato plants (11). Beyer (10, 11) suggested that Ag⁺ interfered with ethylene action by binding to the receptor site which binds ethylene resulting in a reduction of tissue sensitivity to ethylene.

Regulation of ethylene synthesis and its ability to delay senescence has been examined by the use of sodium benzoate, a free radical scavenger (5–7). Baker et al. (6) reported that ethylene synthesis by preclimacteric and postclimacteric avocado and ripe tomato fruits was inhibited in the presence of sodium benzoate. Sodium benzoate in combination with rhizobitoxine extended the life of cut carnation flowers by as much as 86% with minimum damage to the stems and leaves (7). The inhibition of ethylene synthesis by sodium benzoate was attributed to an inhibition of the conversion of ACC to ethylene (4). The purpose of this study was to compare the effect of AVG, Ag⁺, and sodium benzoate on abscission break strength, ethylene production, and loss of Chl and protein in bean explants and to relate these changes to the induction of abscission and senescence.

MATERIALS AND METHODS

Preparation of Bean Explants for Abscission Test. Seeds of Phaseolus vulgaris L. cv Contender were grown in vermiculite for 16 d in a growth chamber under 16 h photoperiod of 1000 ft-c, 25 ± 2°C, and were watered daily with tap water.

Abscission break strength was measured as previously described (23, 24). One-cm petiole sections were excised including the abscission zone adjacent to the leaf blade of primary leaves from bean plants, and were immediately immersed in a test solution which contained either AVG, AgNO₃, or sodium benzoate. After 2 h in the test solution, 20 explants were arranged in 1.5% (w/v) agar plates as previously described (24). The Petri dishes were placed in a plastic box and a stream of moist air was allowed to pass through to prevent desiccation of the explants.

Measurements of Abscission Break Strength. Unless otherwise stated, after 48 h the Petri dishes were removed from the plastic box and the abscission break strength was determined by a method described earlier (24). In control samples, approximately 50% of the explants abscised in a 48-h period as indicated by the time course experiments. Hence, most of the measurements were
made at 24 and 48 h after treatment. Each explant was held at the petiole end with a pair of flat tip forceps with the adaxial side up and the Pulvinar area was pressed against an Ametec force gauge, model L-500. The force required to break the abscission zone was recorded in grams and plotted against the different concentrations of the test solutions. Each experiment was repeated three times with three replications for each and the results are presented as an average of these three experiments.

**Ethylene Measurements.** After 24 h, explants were removed from the agar and the pulvinus was separated from the petiole, placed in 20-ml glass vials and sealed with serum caps for 12 h. A 1-ml gas sample was withdrawn from each vial and injected into a Packard gas chromatograph, model 427, equipped with flame ionization detector and an activated alumina column. Oven temperature was 80°C, the detector 300°C, and the injector 200°C. Results are presented on fresh weight basis with the standard error of the mean.

**Chl Determination.** The petiole and pulvinus of each sample were homogenized separately in a mortar and pestle with 20 ml chilled 80% acetone at 0°C under dim light. The homogenate was vacuum filtered through a Büchner funnel containing Whatman No. 1 filter paper. The filtrate was collected and stored at −40°C until analysis. Total Chl was determined on a Beckman model DB.GT spectrophotometer at 652 nm and the results are presented on a fresh weight basis.

**Protein Determination.** The protein from petiole and pulvinus was measured separately 4 d after treatment by the Bio-Rad protein binding assay (15). Plant samples were homogenized in 20 ml/g fresh weight of 0.1 M K-phosphate buffer (pH 7.2) and centrifuged at 10,000g for 5 min, the supernatant which contains the soluble protein fraction was used for protein estimation, and the results are expressed on fresh weight basis.

**RESULTS AND DISCUSSION**

**Inhibitory Effect of AVG, AgNO₃, and Sodium Benzoate on Ethylene Production.** Application of AVG to bean explants significantly reduced ethylene production in the adaxial pulvinar sections (Fig. 1). Eighty to ninety % inhibition of ethylene synthesis was observed 24 to 48 h after tissue incubation with AVG. In contrast, the proximal petiole sections showed no significant change in ethylene production (data not shown). Poovaiah and Leopold (24) observed similar results with calcium-treated bean explants. AVG concentrations above 1 mm caused tissue damage, and therefore were not included in the tests.

Beyer (9) reported that Ag⁺ interferes with ethylene action at the receptor site, but has no effect on ethylene synthesis. Silver ions added as AgNO₃ have later been shown to inhibit ethylene synthesis, as well as action (3). Our results show that an increase
in AgNO₃ concentration up to 100 𝜇M causes a progressive decrease in ethylene production in the adaxial pulvinar tissues 48 h after treatment (Fig. 2). We have also observed that AgNO₃ concentrations above 100 𝜇M caused tissue browning and increased ethylene synthesis (data not shown).

Inhibition of ethylene production by sodium benzoate (free radical scavenger) was demonstrated by Baker et al. (5, 6) and Apelbaum et al. (4) in avocado, tomato, bean, and peas. Wang et al. (29) and Apelbaum et al. (4) reported that sodium benzoate inhibited ethylene production by interfering with the conversion of ACC to ethylene even in the presence of exogenous ACC. Our results show that infiltration of bean explants with sodium benzoate at concentrations between 50 𝜇M and 5mM reduced ethylene production in the pulvinar sections (48 h after treatment) (Fig. 3). However, ethylene production after 24 h of tissue incubation was either slightly increased or remained unchanged (Fig. 3). Baker et al. (6) reported that sodium benzoate effectively inhibited ethylene production in ripe tomato and avocado fruit tissues whereas AVG did not inhibit.

Although the exact mechanism of inhibition of ethylene synthesis by these chemicals is not clearly known, our results show that AVG inhibition of ethylene synthesis was more effective than that of sodium benzoate and silver nitrate.

Effect of AVG, AgNO₃, and Sodium Benzoate on Leaf Removal Force. AVG, AgNO₃, and sodium benzoate which were found to inhibit ethylene synthesis, were also found to inhibit the abscission process (Fig. 4). Comparing the relative effectiveness of these chemicals at the concentrations used, we have observed that at the lower concentrations there were little differences in the abscission break strength. However, as the concentrations increased sodium benzoate appears to have a better effect without any visible damage to the tissue. When the explants were left on the agar plates for an extended period of time, they abscised irrespective of the treatment. Wang et al. (29) observed that snapdragon florets were less sensitive to sodium benzoate than rhizobitoxine (an AVG analog) treatment, particularly at the early periods of treatment.

Effects of AVG, AgNO₃, and Sodium Benzoate on Chl and Protein Content. Changes in Chl and soluble protein were used as indicators of senescence (22, 24). Chl content in the pulvinar sections were determined after treatment of the explants with AVG, AgNO₃, and sodium benzoate. Figure 5 shows that there were very small differences in Chl content between the treatments. However, when the concentrations of these chemicals increased, sodium benzoate maintained higher amounts of Chl, followed by AgNO₃ and AVG.

Determination of the protein content in the pulvinar sections (Fig. 6) showed a significantly higher amount of protein in AgNO₃-treated explants than AVG and sodium benzoate. In conclusion, our results suggest that AVG, Ag⁺, and sodium benzoate which are potent inhibitors of ethylene synthesis and/or action alter both the senescence induction phase and separation...
tion phase in bean explants.

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LITERATURE CITED