J. Amer. Soc. Hort. Sci. 105(3):304–307. 1980.

Control of Ethylene Synthesis and Action by Silver Nitrate and Rhizobitoxine in Petunia Leaf Sections Cultured *in Vitro*[1]

P. Gavinlertvatana, P. E. Read, and H. F. Wilkins[2]
Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108

Additional index words. tissue culture, *Petunia hybrida*, kinetin

Abstract. **Pretreatment of excised leaves of *Petunia hybrida* L. with $AgNO_3$ permitted shoot formation equal to the control, when explants were cultured on a medium containing 6-benzylamino purine (BA), but increased callus formation, chlorophyll content, and ethylene production. When explants were cultured on a medium containing α-naphthaleneacetic acid (NAA), pretreatment with $AgNO_3$ promoted callus growth, root formation and extension, and ethylene production, but inhibited root hair formation. Pretreatment of explants with $CuSO_4$ suppressed ethylene production and chlorophyll content. Inclusion of $AgNO_3$ partially overcame the effects of $CuSO_4$ alone. When the rhizobitoxine analog, L-2-amino-4-(2-aminoethoxy)-trans-3-butenoic acid (Rh), was used for pretreatment, ethylene emanation was inhibited for 2 or 3 weeks. Number of shoots and roots, root length, root hair and callus formation were not affected by Rh except that callus growth was reduced on a medium containing NAA. NAA (1.0 mg/liter) promoted callus and root formation and induced high levels of ethylene, while kinetin (0.2 mg/liter) stimulated shoot formation but simultaneously induced much lower levels of ethylene.**

Several approaches have been attempted to alter ethylene effects on plants, including inhibition of ethylene synthesis (14, 15, 16, 21, 22), keeping ethylene below biologically active threshold levels with absorbents (17) or hypobaric conditions (7), and preventing ethylene action (5, 8). Both CO_2 (8) and $AgNO_3$ (5) prevent ethylene action, while Rh (16, 21), cobalt ion (Co^{++}) (15), 3,5-diiodo-4-hydroxy benzoic acid (DIHB) (14) and benzyl isothiocyanate (BITC) (22) inhibit its synthesis. Beyer (5) found that Ag^+ applied as $AgNO_3$ inhibited ethylene responses in several plant species. Even though the mode of action of Ag^+ is not well understood, it acts neither as an ethylene competitor as does CO_2, nor as an ethylene scavenger (5). The Ag^+ ion may interfere with ethylene incorporation on the receptor (4, 5, 6), which is believed to be a protein containing metal(s), possibly Cu^+ (1, 5).

Rh limits ethylene synthesis (16, 21) by specific inhibition of the conversion of methionine to ethylene (1, 16), and increases vase life of flowers (2, 12) and storage life of fruits (16).

We demonstrated (10, 24) that dahlia tissues cultured *in vitro* produced ethylene which was significantly associated with callus growth. Even though the gas constantly diffused from the culture vessels (50% reduction after 2 hr), the levels exceeded recognized biologically active ones. Preliminary experiments showed that $AgNO_3$ (25-400 mg/liter), $CoCl_2$ (238.0 mg/liter), DIHB (0.4 – 2.0 mg/liter), BITC (15.0 – 60.0 mg/liter) and Rh (2.0 – 10.0 mg/liter) could not be used effectively in basal medium because of their phytotoxicity and reaction with organic components. However, these chemicals were effective as pretreatment incubation solutions when applied prior to aseptic culture.

Our objectives herein were to investigate the effects of $AgNO_3$ and Rh as pretreatments, to inhibit ethylene responses and to understand the relationship of ethylene to morphogenetic responses of explants cultured *in vitro*.

Materials and Methods

Experiment 1. Petunia plants, University of Minnesota line 77-278, were grown in the greenhouse under natural conditions during May to July 1977 at St. Paul. Leaves were surface disinfected with 95% ethanol for 15 sec, followed by 0.5% (by volume) NaOCl (5% commercial bleach diluted 1:9) with 1% Tween 20 for 15 min. They were rinsed 3 times with sterile water, immediately incubated in sterile water alone or in solutions containing $AgNO_3$ or Rh for 1½ hr, and sliced into small strips (1 × 0.5 cm) for culture. Three pieces were randomly transferred to 125 ml Erlenmeyer flasks containing 50 ml of sterilized modified petunia agar medium (9) and capped with aluminum foil. The basal medium was Murashige and Skoog macroelements (19), Nitsch and Nitsch microelements and vitamins (20) and 0.2 mg/liter BA or 1.0 mg/l NAA. The pH of the medium was adjusted to 5.8 ± 0.1 and 0.8% bacto agar was added prior to autoclaving at 121°C for 20 min. All explants were incubated at 21°C under continuous cool-white fluorescent light at 7.4 $\mu Em^{-2}sec^{-1}$.

Experiment 2. Disinfected leaves of 'Red Magic' petunia, grown under 12 hr light and 12 hr dark cycle of 64 $\mu Em^{-2}sec^{-1}$ light irradiance, supplied by both incandescent and fluorescent lamps, were incubated in sterile water alone or sterile water containing $AgNO_3$, $CuSO_4 \cdot 5\ H_2O$ or combinations of the 2 salts for 1½ hr. They were rinsed with sterile water, and explants were removed and cultured as described above. Ethylene levels were determined at 3 to 7 day intervals by gas chromatography using the same methods as previously reported (10).

In experiment 1, degree of callus formation and growth, root and root hair formation, root growth and shoot number were recorded 10, 17 and 40 days after transfer while callus growth and chlorophyll content were also measured in experiment 2. Callus formation was evaluated visually at 10 and 17 days and the tissues were weighed after 40 days. Callus formation was evaluated from 1 to 4 as follows: 1 = no callus, 2 = small callus formation at the cut edge of the leaf, 3 = large amount of callus at cut edge of the leaf, and 4 = large amount of callus at cut edge and covering the leaf surface. Because roots were relatively short and the area of root hairs difficult to distinguish, root hair formation was scored as follows: 1 = no root hairs, 2 = small number of root hairs at base of roots, 3 = numerous root hairs at base of roots, and 4 = numerous hairs all over the roots. Root length was measured as the longest root on day 10, 17 and 40. Chlorophyll a and b were measured

[1]Received for publication August 7, 1978. Scientific Journal Series Paper No. 10,347, Minnesota Agricultural Experiment Station. Received for publication August 7, 1978.

The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.

[2]Graduate Student and Professors, respectively.

with a Beckman model ACTA III spectrophotometer (Beckman Instrument, Inc., Fullerton, CA 92635). Five flasks per treatment were randomized completely in both experiments.

Results and Discussion

Petunia leaf segments pretreated with $AgNO_3$ (50 or 100 mg/liter) for 1½ hr. showed no phytotoxicity and formed callus within 2 weeks. After 3 months, callus produced by the control turned brown while callus from $AgNO_3$ treatments remained green. $AgNO_3$ pretreatment also increased callus formation when compared to the water control (Table 1).

$AgNO_3$ pretreated explants in experiment 1 formed more callus while Rh pretreated tissues formed the same or less callus than control tissues (Tables 1 and 2). Further, $AgNO_3$ increased root no. and root length, but reduced root hair formation. Rh treatments did not affect root hair formation, root no. or root growth. Explants cultured on a medium containing BA (0.2 mg/liter) formed less callus than those cultured on NAA (1.0 mg/liter) (Table 2). Shoots appeared first on the BA medium, but neither chemical affected shoot number at 40 days (Table 2).

$AgNO_3$ promoted ethylene production in explants cultured on a medium containing NAA or BA, while Rh reduced it for 2 or 3 weeks (Fig. 1). Ethylene production was increased more by NAA than by BA (Fig. 1).

$AgNO_3$ at 25, 50, 100 or 200 mg/liter stimulated chlorophyll a and b formation in experiment 2, but did not affect callus formation. Chlorophyll content was highest at 50 mg/liter $AgNO_3$ (Table 3). Pretreatments of explants with $CuSO_4 \cdot 5\ H_2O$ (50, 100, or 200 mg/liter) reduced callus weight and chlorophyll a and b (Fig. 2 and Table 3). Inclusion of $AgNO_3$ (100 mg/liter) greatly inhibited the effect of $CuSO_4$ in reducing chlorophyll content. As in experiment 1, $AgNO_3$ increased ethylene concentration while $CuSO_4$ decreased it or had no affect. $AgNO_3$ plus $CuSO_4$ increased ethylene concentration slightly (Fig. 3).

Table 1. Effect of pretreatment of leaves with $AgNO_3$ on callus formation in peutnia leaf discs cultured *in vitro*.

$AgNO_3$ (mg/liter)	Mean fresh wt of callus[z] (g)	Mean dry wt of callus[z] (mg)
0	1.748 a[y]	126 a[y]
50	2.189 b	164 b
100	2.417 b	171 b

[z]Mean of 5 replications. Modified Murashige and Skoog medium containing 1.0 mg/liter NAA.

[y]Mean separation, within columns, by Duncan's multiple range test, 5% level.

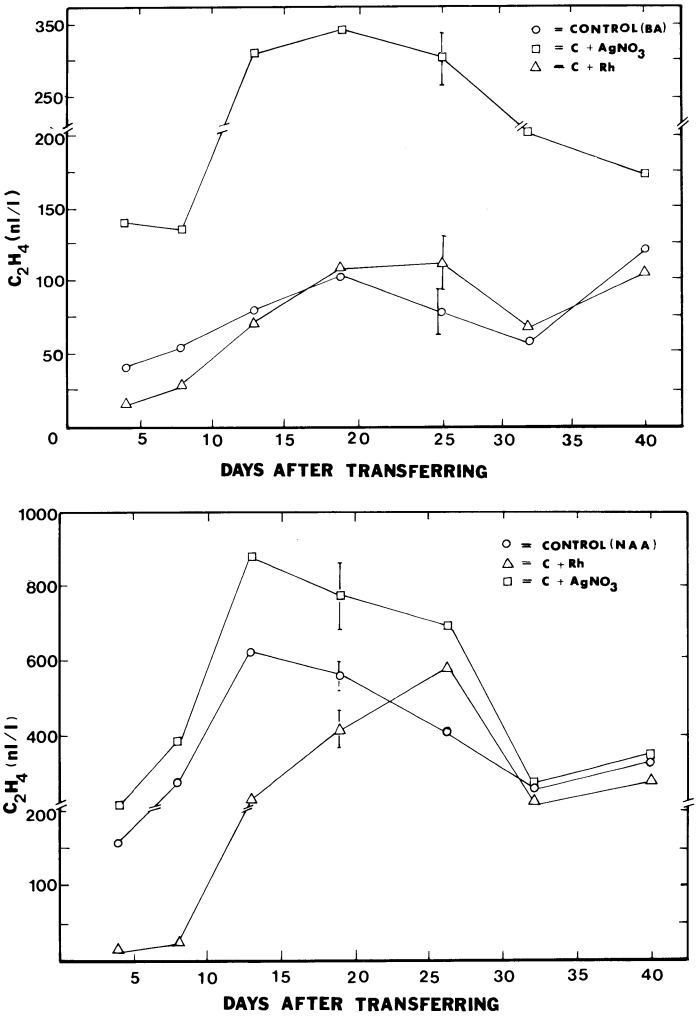

Fig. 1. Effects of $AgNO_3$ and rhizobitoxine analog (Rh) pretreatments of petunia leaves on C_2H_4 emanation by cultured explants. Explants were cultured on a defined medium containing either (top) 0.2 mg/liter BA, or (bottom) 1.0 mg/liter NAA. Each point represents the mean of 4 replications. Φ represents a 95% confidence interval (CI) applied to every point within the treatment. An overalp of CI indicates no significant difference among means at the 5% level.

Table 2. Effects of pretreatment of leaves with $AgNO_3$ or rhizobitoxine analog (Rh) on callus and shoot formation (BA medium), or callus, root number and root growth (NAA medium) in *Petunia hybrida* leaf explants cultured *in vitro*.

Pretreatment[z] (for 1.5 hr)	Concn (mg/liter)	Callus wt (mg) fresh	Callus wt (mg) dry	No. of shoots	No. of roots	Root wt (mg)	Root length (mm)
		BA medium					
Water		2114 a[y]	202 a	4.7 a	--	--	--
Rh	10	2400 ab	212 a	4.7 a	--	--	--
$AgNO_3$	100	2969 b	284 b	4.0 a	--	--	--
		NAA medium					
Water		7540 b	495 a	--	78.7 a	152 a	18.3 a
Rh	10	5758 a	437 a	--	87.7 a	180 a	22.3 a
$AgNO_3$	100	8719 c	574 b	--	117.0 b	334 b	47.7 b

[z]Explants on modified Murashige and Skoog medium containing 0.2 mg/liter BA or 1.0 mg/liter NAA.

[y]Mean separation, within columns and media, by Duncan's multiple range test, 5% level.