Ethylene Levels in Flask Atmospheres of *Dahlia pinnata* Cav. Leaf Segments and Callus Cultured *in Vitro*¹

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Abstract. Biologically active levels of ethylene were accumulated in flask atmospheres of leaf segments and callus of dahlia cultured *in vitro*. The ethylene levels were dependent on concentrations of α-naphthaleneacetic acid (NAA) and 6-furfurylamino purine (kinetin) in the medium. NAA promoted ethylene levels to a greater degree than kinetin. NAA at 1 mg liter⁻¹, but not 5 or 10 mg liter⁻¹, interacted with kinetin to stimulate ethylene synthesis. Reducing ethylene concentrations in the flasks by potassium permanganate absorption had no effect on callus formation from leaf tissue.

Tissue culture success is influenced by several factors, including species variability (9), kinds of tissue (9), nutritional components of the medium (8, 9), growth regulators (9, 11), incubation conditions (9), and gas environments (5, 7, 10). Much tissue culture research has been done on modification of nutritional components and growth regulators in the medium, but little has been studied on flask atmospheric environments. Ethylene produced by plant tissue (1, 12) has been found in culture flask atmospheres (5, 10), but little is known of its effects in tissue culture. LaRue and Gamborg (5) found that ethylene was produced in cell suspension culture, but the amounts were low and evidently did not affect cell growth. This supported the previous report of Mackenzie and Street (7) who applied ethylene in the form of ethephon to sy-camore cell culture. However, Wochok and Wetherell (13) found that ethephon suppressed organized growth in cultivated wild carrot tissue, and Hsu and Stewart (3) found that the ethylene applied to an agar medium inhibited normal differentiation of cotton ovules and promoted callus formation. Their studies were conducted by using a gas-tight container and ethylene was calculated in terms of production per unit of time. However, gas-tight containers are not commonly used in tissue culture, but foam, cotton or aluminum foil covers are normally used. Even though a gas-tight cap is sometimes used, the cap is normally left loose to allow gas diffusion. We believe that the concentration of ethylene to which tissues were subjected and length of exposure affect the physiology and morphology of tissues cultured *in vitro*. Therefore, we investigated the levels of ethylene in the atmospheres of flasks with conventional covers and the influence of such levels on the growth of callus.

Materials and Methods

A modified Murashige and Skoog agar medium (MMS) (8) with 0, 1.0, 5.0, or 10.0 mg/liter NAA and/or 0, 1.0, 5.0 or 10.0

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mg/liter alone or in all combinations was used for these experiments. The pH was adjusted to 5.8 ± 1.0 with 1M NaOH or 1M HCl, and 50 ml aliquots were poured into 125 ml erlenmeyer flasks which were covered with 1 layer of aluminum foil, and autoclaved at 121°C for 10 min. Potassium permanganate (0.5 g) in a small vial was inserted inside half of the culture flasks before autoclaving. After surface sterilization with 0.5% (v/v) NaOCl for 10 min and rinsing 3 times with sterilized deionized-distilled water, leaf explants (1.5 x 0.5 cm) of young fully expanded leaves of *Dahlia Nita*, were cut through the mid-rib and transferred to each flask.

Another experiment was conducted by using callus, which had previously been cultured on an agar medium containing 5 mg/liter of both NAA and kinetin. This callus was cut into pieces and 100, 200, 300 or 400 mg callus explants were transferred onto agar medium containing 5 mg/liter of both NAA and kinetin.

In a subsequent experiment, 3 pieces of callus (lg total weight) or 3 fresh leaf segments were placed on a MMS medium containing 5 mg/liter kinetin and 0.5 or 5 mg/liter NAA in 125 ml erlenmeyer flasks. For both issues, KMnO₄ crystals were suspended in cheesecloth above medium and tissue in half of the flasks. Treatments in each experiment were replicated 5 times with 1 flask each. Each experiment was repeated at least once with similar results.

Callus formation from the leaf segments was scored from 1 to 5 as follows: 1 = no callus, 2 = small amount of callus at the cut edge of the leaf, 3 = large amount of callus at the cut edge of the leaf, 4 = large amount of callus at the cut edge of the leaf and small amount of callus on the leaf surface, and 5 = large amount of callus at both cut edge and leaf surface.

Ethylene levels were determined using a Varian model 1440 gas chromatograph with a flame ionization detector. The column was 3.2 mm I.D. x 45 cm, packed with activated alumina 60/80 and maintained at 70°C. A 600 microliter air sample from the culture flask was withdrawn for analysis with a 1 ml gas-tight syringe (Precision Sampling Corp.). The flasks were resealed with another layer of aluminum foil to prevent contamination.

A preliminary experiment using known ethylene levels added to the culture flasks revealed that ethylene loss through the foil cap was 50% after 2 hr. This rate of loss was constant and we recognized this fact in discussing our data.

**Results and Discussion**

Ethylene production was stimulated by 5.0 or 10.0 mg/liter NAA while kinetin had little or no influence on ethylene production (Fig. 1A). A significant interaction was observed between NAA at 5 or 10 mg/liter and all kinetin levels. A similar ethylene response from etiolated mung bean hypocotyls has been reported (4, 6). NAA at 1 mg/liter caused little ethylene increase and had no interaction with kinetin (Fig. 1A). Five and 10 mg/liter of NAA and kinetin caused greatest ethylene accumulation. Significantly lower levels of ethylene were detected when no growth regulators, kinetin alone or kinetin combined with NAA at 1 mg/liter (Fig. 1A) were used. The KMnO₄ absorbed up to 80% of the total ethylene and lowered the ethylene level to 25 nl/liter.

Callus formation was observed on all media containing NAA and kinetin whether KMnO₄ was present or not (Fig. 1B). When KMnO₄ was not present, callus formation was correlated with ethylene levels (r=0.843), but if KMnO₄ was present, callus formation was not correlated with ethylene levels (r=0.231). Therefore, callus formation is dependent on the concentration of NAA and kinetin.

When various amounts of dahlia callus were used, ethylene levels were directly proportional to amounts of tissue (Fig. 2). The larger the callus, the higher the ethylene levels measured. The ethylene levels were increased by about 1 nl/liter–mg of cal-

![Fig. 1. Effect of NAA and kinetin on ethylene levels in flask atmospheres (A) and callus formation (B) from dahlia leaf segments cultured in vitro.](image-url)
Fig. 2. Effect of initial amount of callus on ethylene emanation and callus growth. Data taken 28 days after transfer.

Ethylene levels seem to be a result of callus formation rather than the cause. However, ethylene effects if any, may occur quickly before absorption by KMnO₄, or KMnO₄ may only absorb excess ethylene. Also, ethylene may not affect callus formation but rather affect other developmental processes such as callus growth or differentiation (4, 12).

High concentration of NAA (5 mg/liter) significantly promoted ethylene production more than 0.5 mg/liter NAA in both leaf and callus tissues. The KMnO₄ absorbed 30 to 60% of total ethylene with the leaf tissue, but callus formation and callus growth were similar to the control (Fig. 3 & 4).

For leaf segments, the ethylene increased over time with significantly greater levels at 5 mg/liter NAA than at 0.5 mg/liter. Ethylene levels increased slightly the 3rd week after transfer which was concurrent with the first visible callus formation (Fig. 3). Levels then decreased and remained unchanged during the 4th week. Concomitantly with anthocyanin-like pigment development, ethylene levels increased dramatically during the 6th week. These changes in ethylene level may be associated with the rate of cell division (7) and subsequent development.

With callus, the ethylene levels were also dependent on the concentration of NAA, the higher the concentration of NAA, the higher the ethylene level (Fig. 4). Ethylene level essentially remained constant during the first 2 weeks, but then increased and fell off by the fifth week. This curve may be associated with callus

Fig. 3. Effect of NAA and KMnO₄ on ethylene in flask atmosphere from dahlia leaf tissue cultured in vitro. C denotes point at which callus first visible; A is the point at which anthocyanin pigments were first observed in all 4 treatments. Each point represents a mean of 4 replications. Φ represents a 95% Confidence Interval applied to each data point within a treatment.

Fig. 4. Effect of NAA and KMnO₄ on ethylene in flask atmosphere from callus tissue incubated over a 5-week period. Each point represents a mean of 4 replications. Φ represents a 95% Confidence Interval applied to each data point within a treatment.

growth and senescence as described for CO₂ and ethylene emission from senescent carnation flowers (12) or climacteric fruit (2). These results suggested that ethylene levels are associated with tissue transition and increases in callus growth.

Literature Cited


Water Stress, Growth, and Critical Water Potentials of Rabbiteye Blueberry (Vaccinium ashei Reade)1

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Abstract. Moderate and severe water-stress, as determined by decreases in stomatal conductance, resulted in significant reduction of leaf area and plant weight in 1-year-old containerized 'Bluegem' rabbiteye blueberry. Drought-tolerance appeared to be intermediate to other plant species based on a number of physiological factors. Critical water potential for stomatal closure was −2.2 MPa, transpiration ratio averaged 222 g of water transpired per g dry matter produced and relative water content changed 6.4% per 1.0 MPa change in water potential.

Established rabbiteye blueberry bushes have been observed to be relatively drought-tolerant (1, 4). A waxy leaf cuticle which may partially occlude the stomatal antechamber (1), and moderately low stomatal conductances limit water losses during drought (6). Leaf transpiration ratio is low relative to other mesophytes (16) and rabbiteye blueberries remain productive even after exposure to severe spring drought periods (1, 4). Conversely, newly planted or container-grown bushes appear drought-sensitive. Spiers (15) found that 1- and 2-year-old 'Tifblue' bushes had a higher survival percentage and better growth under irrigated as compared with non-irrigated conditions.

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Stomatal response to water deficits, and particularly, the critical water potential for stomatal closure are important factors associated with drought-tolerance (17, 18). Stomata tend to close at much lower water potentials in drought-tolerant than in drought-sensitive plants, which allows for photosynthesis over a wider range of water deficits. Differences in stomatal conductance and overall drought-tolerance have been observed among rabbiteye blueberry cultivars in the field (1, 6); however, critical water potential for stomatal closure has not been determined for rabbiteye blueberry. The relationship between relative water content (RWC) of the leaf and water potential also may be an indicator of plant drought-tolerance (11, 14). Bannister (2) found that Vaccinium myrtillus L. could withstand RWC as low as 68% without serious damage. This relationship has not been studied in cultivated Vaccinium species. The objectives of this study were to investigate the relationship of critical water potential and RWC to rabbiteye blueberry drought-tolerance as well as the effects of moderate and severe water-stress on growth of containerized rabbiteye blueberry plants.