VOLATILE EMISSIONS OF PLANT TISSUE CULTURES
I. Identification of the Major Components

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(Received September 15, 1978; accepted February 1, 1979)

SUMMARY

The low-molecular-weight volatiles released by a variety of plant tissue cultures were examined by gas chromatography. Callus cultures invariably produced carbon dioxide, ethylene, acetaldehyde, and ethanol. In cultures with developed shoots, ethanol was absent and acetaldehyde was detected only rarely.

Key words: carbon dioxide; ethylene; acetaldehyde; ethanol; plant tissue culture.

INTRODUCTION

During the course of development in vitro, plant tissues not only deplete the nutrients that are furnished in the medium, but also release substances that can accumulate in the cultures. These substances, some of which may have profound physiological effects on the cultured tissues, include volatile and nonvolatile compounds. This research was focused on the volatile products that accumulated in a variety of plant tissue cultures. Identifications were made of the low-molecular-weight emanations that were ubiquitous or encountered in substantial quantities. Their physiological significance and factors that might influence their synthesis and release remain under investigation.

Autogenous volatile growth factors have been reported in cell suspension cultures. In Acer pseudoplatanus L. cultures, growth was enhanced by a substance that was absorbed by KOH but could not be replaced by carbon dioxide (1). The growth inhibition by an unidentified factor produced by cultures of Atropa belladonna was not attributable to oxygen depletion or toxic levels of carbon dioxide or ethylene (2). Talbot and Street (3) reported enhanced growth of cultured wheat roots in the presence of an unidentified volatile root metabolite. The substance was not absorbed by mercuric perchlorate, indicating that it was not ethylene. Ethylene production is common to a number of plant cell cultures (4, 5). Recently, ethanol was shown to be a product of excised citrus ovules (6). Volatiles of higher molecular weight, such as terpenoids and volatile oils, also have been reported in some tissue cultures, e.g. in Andrographis paniculata (7) and Ruta graveolens (8).

MATERIALS AND METHODS

Tissue culture. Cultures were grown in 25- by 150-mm glass culture tubes, each containing 25 ml nutrient agar and capped with a polypropylene closure (Kaput, Bellco). Four types of cultures were examined: (a) callus only; (b) cultures with fully developed shoots, identified in the data as "proliferating shoot cultures"; (c) "woody nodal explants," excised to include the nodal region of a woody stem with an unemerged bud; and (d) "herbaceous nodal explants" from herbaceous species that have proved difficult to culture in vitro. The Daucus and Nicotiana callus cultures were grown in the dark. All other cultures were maintained at 27°C under a daily regime of 16 hr illumination with 1000 lx from Gro Lux lamps. The nutrient media varied with the species and type of tissue being cultured. All cultures were allowed to develop for at least 2 weeks within the passage before sampling for gas analysis. Triplicate cultures were sampled. No ethanol was used in medium preparation or in disinfection of tissues or instruments.

Gas sampling. Cultures were prepared for gas sampling by replacing the polypropylene Kaputs...
(Bellco) with rubber vaccine caps or Kaputs that had been modified for gas sampling. The modification of the Kaput involved a 2-mm perforation at its top center, sealed with two layers of builders' duct tape (Frost King; Thermwell Products Co., Inc., Los Angeles, California). Flaming of the culture tube rim was omitted since this caused evolution of methane and other volatiles from the vaccine caps. Gas samples were obtained by inserting a syringe needle through the vaccine cap or the duct tape seal. Glass tuberculin syringes (B-D Glaspak, 1-cc), with Yale 22 stainless-steel needles were used to remove the gas. Samples were obtained when 9 to 12 hr of the light cycle had elapsed; times between preparation for sampling and sample extraction are given in the Results.

Gas analysis. The procedure of Negm, Smith and Kumamoto (9), which is restrictive (under the conditions used) to low-molecular-weight volatiles, was employed. Gas samples (0.5 ml) were analyzed on a Beckman GC-4 dual hydrogen flame gas chromatograph with a 285- by 0.32-cm stainless-steel column packed with 50- to 80-mesh Porapak Q (Waters Associates Inc., Milford, Massachusetts). Column eluates were passed, via a thermal conductivity detector to monitor CO2, to the hydrogen flame ionization detector to determine the other gases. The concentrations of carbon dioxide, ethylene and ethane were determined by calibration with standard gas mixtures. Ethanol and acetaldehyde were identified by co-chromatography with standards at 78° and 100° C, and the quantities in the gas phase within the cultures (volume taken as 30 ml) were estimated from calibration curves established from aqueous solutions of standards. After completion of gas analysis, plant tissues were weighed and gas values were adjusted to a 1-g fresh-weight basis. Ethylene values were corrected to compensate for loss of approximately 6% per day from vaccine-capped tubes.

RESULTS AND DISCUSSION

Gases detected and identified from tissue cultures were carbon dioxide, ethylene, ethane, acetaldehyde and ethanol, with gas chromatographic retention times at 78° C of 1.3, 1.9, 2.3, 18.5 and 58 to 60 min, and at 100° C of 1.0, 1.3, 1.6, 8.3 and 19.7 min. Nutrient media alone showed no emission of volatiles. Daucus and Lactuca callus sometimes also showed a small unidentified peak (possibly formaldehyde or methanol) immediately preceding the acetaldehyde. Volatiles determined in cultures 1 day after closing tubes with vaccine caps are shown in Table 1.

Carbon dioxide levels tended to be lowest in cultures with well developed shoots (Table 1B). Some of these cultures, such as Clerodendrum and Dicksonia, maintained a virtually carbon-dioxide-free atmosphere in the light. Since these cultures accumulated carbon dioxide in the dark, the low levels in light are presumably a result of photosynthetic utilization.

Ethylene production showed a variable pattern, with the highest levels in two of the callus cultures, Nicotiana and Phoenix (Table 1A). The rate of ethylene production was low for Nicotiana callus (19.8 nmol per g fresh weight per day) compared with values for Glycine (123.1 nmol per g fresh weight per day) and Rosa (438.01 nmol per g fresh weight per day) calculated from maximum rates obtained in suspension cultures by other investigators (5). The average ethylene production in cultures with shoots was 0.74 nmol per g fresh weight per day, which may be compared with a rate of 0.43 nmol per g fresh weight per day from etiolated pea hypocotyl tissue calculated from data of Burg and Burg (10). Ethylene production was not detected in Adiantum cuneatum and Ficus benjamina cultures; the amounts observed did not exceed the background level of 0.04 ± 0.02 ppm.

The ethane levels, observed frequently, approximated those in room air (0.05 ± 0.06 ppm). Acetaldehyde and ethanol, absent from most cultures with shoots, were characteristic of the callus cultures and the woody nodal explants (Table 1C).

The acetaldehyde and ethanol cannot be attributed to increased levels of carbon dioxide. The highest levels of accumulated carbon dioxide (as evident in Apium and Daucus callus cultures) were associated with a relatively low content of acetaldehyde and ethanol. In the cultures with shoots, none of which yielded ethanol, the levels of carbon dioxide sometimes exceeded those observed in callus cultures.

The herbaceous nodal explants (Table 1D) represented cultivars which, under the culture conditions used, failed to manifest bud emergence or callus formation and died within a relatively short period (about four weeks). These explants showed high levels of CO2 and ethylene production compared to most cultures with profuse shoot development. Ethanol was present in Pelargonium (but not in Begonia) cultures.