The Effect of Salt Concentration on Auxin Stability in Culture Media

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Short Communication

The Effect of Salt Concentration on Auxin Stability in Culture Media

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ABSTRACT

The concentrations of indole-3-acetic acid (IAA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) were followed for 35 days in cell-free liquid medium containing 100, 50, or 0% Murashige-Skoog (MS) salt base. Although the concentrations of NAA or 2,4-D remained constant the level of IAA decreased to only 11% of the original concentration after 35 days in the presence of 100% MS salt base. The observed rate of IAA degradation was accelerated by the presence of MS salts.

In vitro plant culture has evolved from attempts to reduce the complexity of the plant system so that growth and development processes can be more easily studied. Although the 'controlled' culture systems are simpler than natural environments, the level of complexity is still sufficient to introduce a substantial level of variability. Over the years, numerous media compositions have been developed to elicit selected plant responses (1, 2, 4). Great interest and care have been given to the preparation of culture media; however, limited data are available on changes in media composition during incubation.

Auxins are a critical component of artificial culture systems but little is known about the factors affecting their persistence during incubation. Synthetic auxins such as 2,4-D have been reported to be more stable than the natural auxin, IAA (4, 5, 11). Studies with cell suspensions of *Acer pseudoplatanus* have described the loss of 2,4-D from media as a result of glycosylation (6, 7). However, the rate and degree of glucosylation were highly variable. The tissue response elicited by the presence of auxin varies according to both concentration and chemical structure (3, 10, 12). To exploit fully the potential of auxins in tissue culture, we must develop a better understanding of how to regulate their presence in artificial growing systems. This can only be accomplished by separating the loss of auxin that occurs in sterile medium alone from the loss associated with cellular metabolism.

A study was designed to follow changes in auxin concentrations as a function of salt concentration in artificial media. The investigation focused exclusively on the contribution of the physical or nonbiological component of culture media to the degradation pattern of several auxins commonly used in tissue culture.

MATERIALS AND METHODS

Culture. Each of the auxin treatments was developed by adding 3 mg/L of 2,4-D (1.4 mM), IAA (1.7 mM) or NAA[1] (1.6 mM) to liquid medium containing 100, 50, or 0% (distilled H_2O) of the recommended MS salt base and 20 g/L of sucrose (8). Nine auxin-MS media combinations were evaluated in triplicate. Each experiment was conducted twice.

Following mixing of the media constituents, pH was adjusted to 5.6 with 1 N NaOH. One hundred ml of each medium were dispensed into 250-ml screw top glass Erlenmeyer flasks and autoclaved for 20 min at 121°C and 1.03×10^5 Pa. The sterilized media were incubated in an environmental incubator/rotary shaker (New Brunswick Model G24)[2] at 150 rpm and 30 ± 1°C. Parts of the shaker were covered with black paper to eliminate any light during incubation. Within 24 h of preparation and at weekly intervals for the following 35 d, a 5-ml aliquot was aseptically drawn from each flask for analysis. Flasks were visually monitored and subsamples periodically assayed on solid medium for contamination. All contaminated flasks and the associated data were discarded.

Sample Analysis. Subsamples of culture media containing either IAA or NAA were analyzed immediately after filtration through a 0.45 μm filter. Subsamples containing 2,4-D were adjusted to pH 3 and partitioned into diethyl ether to eliminate interference in the sample. The ether was evaporated to dryness and the sample dissolved in a small volume of methanol. Samples were analyzed by reverse phase HPLC. A Laboratory Data Control (Riviera Beach, FL) HPLC equipped with dual pumps, Rheodyne injector (20 μl sample loop), spectroMonitor III variable wavelength detector and automatic sampling system was used. Conditions for analysis consisted of a Spherisorb ODS 5 μm column (0.4 × 25 cm) eluted isocratically with 25% (IAA and NAA) or 40% (2,4-D) acetonitrile and water (v/v) adjusted to pH 3 with phosphoric acid. Auxin concentrations were determined by reading absorbances at 280 nm. Under the conditions of this analysis, the lower limit of detection for IAA was 2 ng.

Data Analysis. Peak areas were computed by integration and quantified by comparison to known concentrations of authentic auxins. Treatments were evaluated using analysis of variance (ANOVA) and regression analysis. Least significant differences

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[1] Abbreviations: NAA, naphthaleneacetic acid; MS, Murashige-Skoog.

[2] Mention of companies or trademark does not imply endorsement by the United States Department of Agriculture or Texas Agricultural Experimental Station over similar products from other commerical sources.

(LSD) were computed for each treatment at the 0.05% level of confidence.

RESULTS AND DISCUSSION

The analyses of 2,4-D and NAA in 100% MS indicated that no detectable losses occurred over the 35-d test interval (Fig. 1). However, IAA proved to be much less stable than 2,4-D or NAA, its concentration declining to approximately 11% of the original within 35 d.

The auxin losses were initially determined in the presence of 100% MS salts, standard cell culture components. The MS salt base is a complex mixture of inorganic ions potentially capable of interacting with IAA. Therefore, auxin degradation curves were developed for liquid media containing 50% and 0% MS salts. The stabilities of 2,4-D and NAA were unaffected by the reduction of MS salts in the medium (Figs. 2 and 3). Degradation of the less stable auxin, IAA, was substantially slower in medium containing reduced levels or lacking MS salts. The amount of IAA remaining in a medium with 50% MS salts after 21 d was 33% higher than levels in 100% MS salts incubated over the same period. The percentage of IAA remaining in the 0% MS salts treatment was twice the amount found in the medium
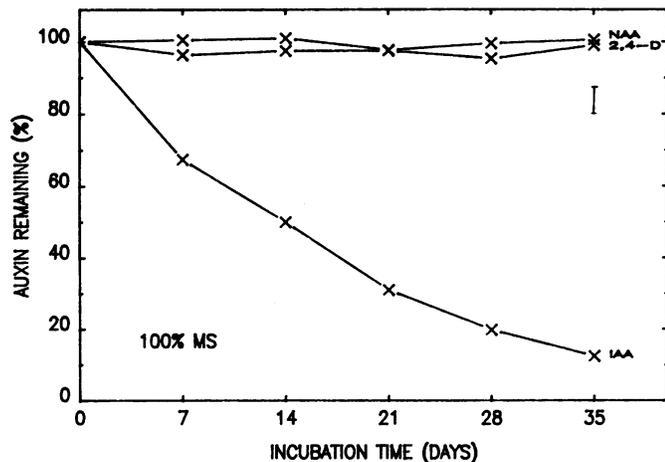

FIG. 3. Percentage of 2,4-D, NAA, and IAA remaining at 7-d intervals during incubation for 35 d in 0% MS culture medium. Initial concentrations of each auxin were 3 mg/L. Vertical bars represent LSD at $\alpha = 0.05$.

FIG. 4. The correlation between loss of IAA and incubation time at 0 ($\Diamond$), 50 ($\triangle$), and 100% ($\square$) MS salts. Initial concentrations of each auxin were 3 mg/L.

FIG. 1. Percentage of 2,4-D, NAA, and IAA remaining at 7-d intervals during incubation for 35 d in 100% MS culture medium. Initial concentrations of each auxin were 3 mg/L. Vertical bars represent LSD at $\alpha = 0.05$.
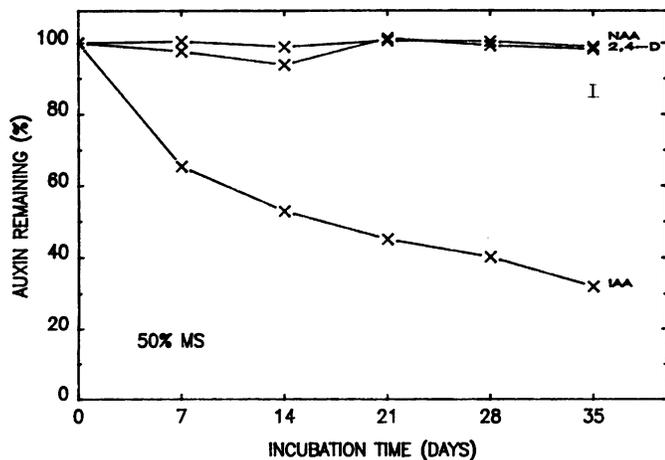

FIG. 2. Percentage of 2,4-D, NAA, and IAA remaining at 7-d intervals during incubation for 35 d in 50% MS culture medium. Initial concentrations of each auxin were 3 mg/L. Vertical bars represent LSD at $\alpha = 0.05$.
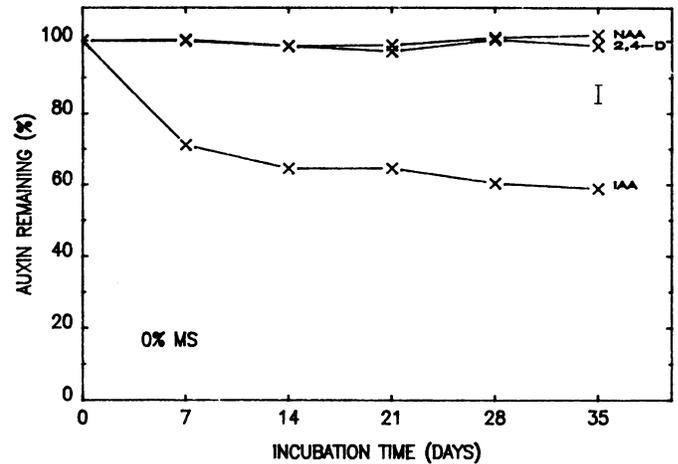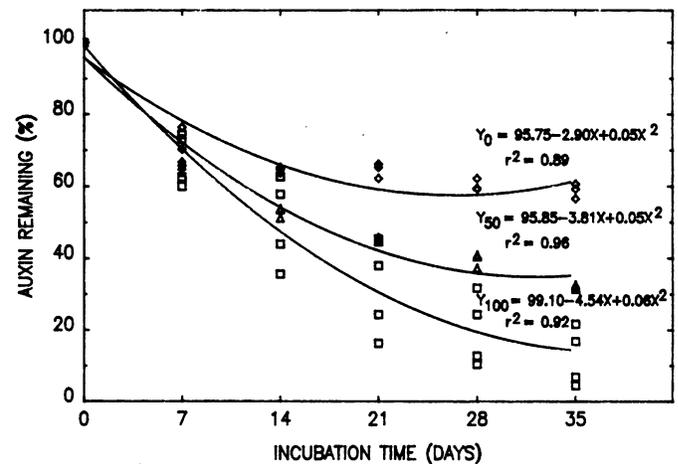
containing 100% MS salts. The initial concentrations of IAA were unaffected by autoclaving of the media which confirmed similar observations reported by Pence and Caruso (9).

The degradation rate of IAA was examined using regression analysis (Fig. 4). The degradation rate over time was significantly different ($\alpha = 0.05$) for all media treatments. The results indicate that mathematical models could be developed to describe the decay of IAA in any culture medium and estimate the loss associated with abiotic degradation of IAA over time.

In our study, the stability of NAA was found to equal that of 2,4-D. However, tissue responses to NAA and 2,4-D at equivalent concentrations can vary dramatically. For example, 2,4-D stimulated callus formation and somatic embryogenesis in *Pharbitis nil* stem explants (10). In the same experiment, IAA stimulated extensive rooting with very little callus formation when tested at concentrations equal to 2,4-D. Differentiation of radicles by *Pharbitis nil* embryos was promoted by NAA, inhibited by 2,4-D and unaffected by IAA. Induction of auxin-habituated tobacco callus was accomplished by culturing the tissue on 1 mg/L of 2,4-D for 35 d (11). A similar response was obtained with an NAA supplemented medium, but only when the concentration was increased by 10-fold over the level required with 2,4-D. Concentration ranges of IAA from 0.01 to 100 mg/L were unable to induce habituated callus tissue.

Morphogenic responses of tissue in culture have been attrib-

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uted to the type of auxin and concentration used in the culture medium. However, the actual level of a particular endogenous or exogenous auxin has seldom been measured continuously over the entire length of the subculturing period. Without knowing the actual endogenous and exogenous auxin levels over time, morphogenic responses of tissue *in vitro* to phytohormone treatments remains more speculation than fact.

The qualitative/quantitative interactions between an auxin and the responding tissue demand that the dynamics of auxin levels be characterized. Background or abiotic losses of the naturally occurring auxin, IAA, may be substantial in some *in vitro* systems independent of the amount of cells present in the medium. The rather dramatic effect of salt concentration on IAA stability represents only a simple example of the poor understanding we have about a system that is assumed to be 'well controlled.' In order to move from phenomenology to an understanding of cause and effect, interactions between the chemical components as well as the biological must be investigated. This study undertakes an initial step in that process.

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