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VOLATILE EMISSIONS OF PLANT TISSUE CULTURES

II. Effects of the Auxin 2,4-D on Production of Volatiles in Callus Cultures

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SUMMARY

The levels of ethanol present in the gas phase of callus cultures were elevated by 2,4-D. Whereas carbon dioxide, ethylene and acetaldehyde also were affected, their changes in relation to auxin were inconsistent and usually smaller in magnitude than that of ethanol. Under conditions independent of auxin concentration, embryogenesis in *Daucus carota* and *Phoenix dactylifera* showed an inverse relationship with the concentration of ethanol in the cultures.

Key words: auxin; ethanol; embryogenesis; plant callus culture.

INTRODUCTION

A study of low-molecular-weight volatiles produced by plant tissue cultures (1) revealed that ethanol (CH_3CH_2OH) and acetaldehyde (CH_3CHO), undetectable in cultures with developed shoots, were common products of callus cultures. The enzyme alcohol dehydrogenase (ADH) is considered to be ubiquitous in plants (2) and presumably serves inter alia in adapting to conditions of respiratory stress. The activity of ADH in a developing plant varies greatly with the stage of development and with the organ studied (3). In peas, seeds and 1- to 2-day-old seedlings show high levels which decline during the major growth period and subsequently rise during the final weeks of plant maturation. Certain organs, such as root nodules, are characterized by marked elevation of ADH activity.

Auxin plays a major role in growth and differentiation of callus cultures. A practical rule of thumb holds that callus cultures established and maintained in media containing auxin may undergo organogenesis or somatic embryogenesis on transfer to auxin-free medium (4–7). Accordingly, we have examined the relationship between the auxin 2,4-dichlorophenoxyacetic acid (2,4-D) and the production of volatiles in callus cultures in an attempt to elucidate the mechanism by which auxin suppresses organized development.

MATERIALS AND METHODS

Callus cultures. Callus cultures of *Apium graveolens* L. var. *dulce*, *Daucus carota* L. var. *sativus*, *Lactuca sativa* L. var. *capitata*, *Nicotiana tabacum* L. 'Wisconsin 38,' and *Phoenix dactylifera* L. were cultured in media described below, and then recultured in media containing varying amounts of 2,4-D, in order to examine the relationship between the auxin and volatile emissions.

The *A. graveolens* cultures, derived from seedling hypocotyls, were grown in the medium of Williams and Collin (8). *D. carota* callus was maintained in a medium by Tisserat and Murashige (9) but modified by increasing the 2,4-D concentration to 0.3 mg per l. Hypocotyl-derived callus of *L. sativa* was cultured in a medium with the following formulation: Murashige and Skoog salts plus (in mg per l) sucrose (30,000), $NaH_2PO_4 \cdot H_2O$ (170), thiamine·HCl (0.4), *i*-inositol (100), 2,4-D (0.1), kinetin (1.0), and adenine sulfate·$2H_2O$ (80). Stem callus of *N. tabacum* 'Wisconsin 38' was grown in a medium similar to that of *Lactuca*, with the addition of 100 mg per l L-tyrosine and the replacement of kinetin by 3 mg per l N^6-isopentenyladenine. *P.*

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dactylifera callus derived from embryos was cultured in a medium containing Murashige and Skoog salts plus (in mg per l) thiamine·HCl (0.4), 2,4-D (100), N^6-isopentenyladenine (1), and Sigma activated charcoal (3000). All media contained 0.8% washed agar (Phytagar) and were dispensed in 25- by 150-mm glass culture tubes in 25-ml aliquots. The tubes were capped with polypropylene closures (Kaputs, Bellco).

In *Daucus* and *Phoenix*, attempts were made to detect correlations between somatic embryogenesis and volatile emissions. Similarly, in *Lactuca*, relationships between shoot bud production and volatile emissions were examined.

Culture conditions. Cultures were grown at 27° C in constant darkness (*Apium*, *Daucus*, *Lactuca*), or under a daily regime of 16 hr exposure to 1000 lx illumination from Gro Lux lamps (*Nicotiana*, *Phoenix*). No alcohol was used in disinfestation of tissues or surgical instruments.

Gas sampling. Cultures were prepared for sampling by replacing the polypropylene Kaputs (Bellco) with Kaputs that had been modified for gas sampling. The modification, in the top of the Kaput, involved a 2-mm perforation which was sealed with two layers of builders' duct tape (Frost King; Thermwell Products Co., Inc., Los Angeles, California). Gas samples were obtained by inserting a syringe needle through the duct tape seal. Syringes (B-D Glaspak, 1-cc) were equipped with Yale 22 stainless-steel needles.

Gas analysis. The procedure of Negm, Smith and Kumamoto (10) was used. 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 carbon dioxide, to the hydrogen flame ionization detector to determine the other gas components. The concentrations of carbon dioxide and ethylene were determined by calibration with standard gas mixtures. Ethanol and acetaldehyde were identified by cochromatography with standards at 78° and 100° C; and the quantities in the atmosphere of the culture tubes, volume taken as 30 ml, were estimated from calibration curves established using aqueous solutions of standards. After completion of gas analysis, plant tissues were weighed, and numbers of somatic embryos or shoots were recorded. Gas values were adjusted to a basis of 1-g fresh weight. Comparisons between treatment means were made using the Student *t*-test (11). Culture media, Kaputs and duct tape produced no detectable volatiles.

Results and Discussion

The volatiles produced by callus cultures in response to 2,4-D can be seen in Table 1. The results, expressed as percentages of control values, showed consistent and often massive elevation of ethanol and acetaldehyde levels ($t=0.1$ or lower). Other parameters examined, i.e. fresh weight and carbon dioxide and ethylene levels, responded variably to auxin. Only in *Nicotiana* callus was the increase in ethylene emanation proportionately greater than the elevation of ethanol. Ethylene levels were suppressed in *Apium* and *Daucus* and unaffected in *Phoenix*. Depression of ethylene levels by 2,4-D has been reported for *D. carota* L. 'Queen Ann's Lace' (12). Interestingly, in *Nicotiana* the auxin indole-3-acetic acid produced effects that paralleled 2,4-D responses with respect to ethylene, acetaldehyde and ethanol

TABLE 1

Effect of Auxin on Fresh Weight and Volatiles From Callus Cultures, Expressed as Percent of Controls Without Added Auxin

Callus Species	Auxin and Concen.		Culture Age	Fresh Wt.	CO_2	C_2H_4	CH_3CHO	CH_3CH_2OH
	mg/l		*days*					
Apium graveolens var. *dulce*	2,4-D	0.5	21	73.04	114.04	48.57	126.03	170.15
Daucus carota L. var. *sativus*	2,4-D	0.3	12	75.47	53.73	87.50	135.68	130.28
Lactuca sativa L. var. *capitata*	2,4-D	0.1	20	202.74	128.77	292.10	315.49	249.85
Nicotiana tabacum L. 'Wisconsin 38'	2,4-D	3.0	14	47.06	211.55	2665.21	804.16	1234.55
Nicotiana tabacum L. 'Wisconsin 38'	IAA	30.0	14	103.92	65.05	552.17	1350.00	1793.19
Phoenix dactylifera L.	2,4-D	30.0	53	40.04	238.79	112.00	200.16	276.32

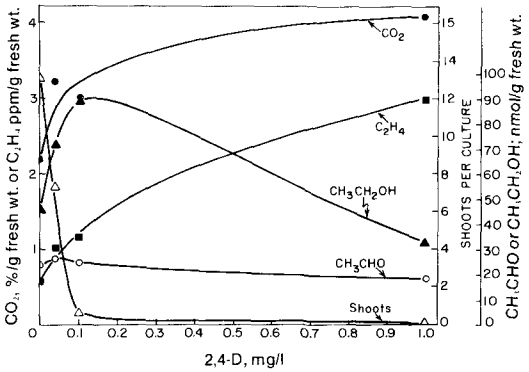

FIG. 1. Effect of 2,4-D on volatile emissions and shoot formation in *Lactuca sativa* callus cultures. Culture ages at gas sampling and at shoot count were 10 and 30 days, respectively.

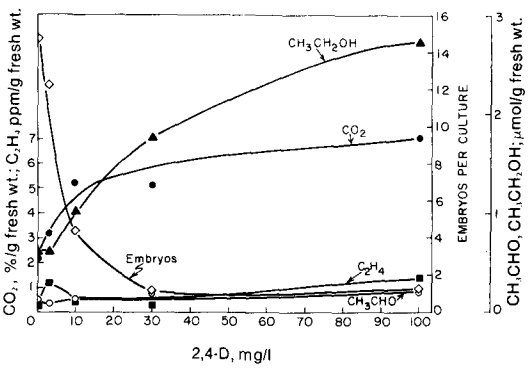

FIG. 2. Effect of 2,4-D on volatile emissions and embryogenesis in *Phoenix dactylifera* callus. Culture ages at gas sampling and embryo count were 53 and 60 days, respectively.

evolution, but not in fresh weight and carbon dioxide release. Ethylene production by apple fruit tissue is stimulated or inhibited by indole-3-acetic acid depending on the stage of maturity of the fruits (13).

The relationships between auxin and concentration, volatiles and organogenesis are shown for *Lactuca* and *Phoenix* in Figs. 1 and 2. Both showed inhibition of organogenesis by auxin. In *Lactuca*, maximum ethanol production was observed in 0.1 mg per l 2,4-D, a concentration that caused almost complete inhibition of shoot formation; both carbon dioxide and ethylene increased with the auxin level. In *Phoenix* (Fig. 2), the most conspicuous response among the volatile emissions was the rise in ethanol. Ethylene showed little change.

In attempts to elucidate possible relationships between ethanol and organogenesis, independent of auxin concentration, variations in organogenesis as related to different culture strains (*Daucus*) or individuals within treatments (*Phoenix*) were examined. Onset of somatic embryogenesis in callus of *Daucus* on transfer to auxin-free medium is a well documented phenomenon (12). Table 2 shows that strain A, a nonembryogenic strain in the auxin concentration used, produced almost twice as much ethanol as strain B, a highly embryogenic line. Since auxin elevates ethanol in carrot callus cultures (Table 1) and exogenous ethanol reversibly inhibits embryogenesis in wild carrot callus (9), the results are consistent with a relationship between endogenous ethanol and somatic embryogenesis. It is interesting that a qualitative change in respiration, involving increased cyanide sensitivity, has been reported to be associated with induction of somatic embryogenesis (*Daucus*) cultures (14).

In *Phoenix* (Table 3), the differences in intensity of somatic embryogenesis in callus cultures within any auxin treatment were correlated with variations in volatile emissions. Cultures with one or no embryos consistently produced more ethanol and less ethylene than those with 5 to 16 embryos. These observations suggested the possibility that the removal of ethanol, perhaps by

TABLE 2

VOLATILE EMISSIONS FROM TWO STRAINS OF CARROT CALLUS WITH DIFFERENT SENSITIVITY TO EMBRYOGENIC SUPPRESSION BY 2,4-D[a]

Strain	Embryos per Culture	Fresh Wt.	Volatiles/g Fresh Weight			
			CO_2	C_2H_4	CH_3CHO	CH_3CH_2OH
		g	*%*	*ppm*	*nmol*	*nmol*
A	0	0.391±0.166	4.27±2.37	0.09±0.06	13.80±4.07	39.64±11.55
B	18.75±4.03[b]	0.248±0.028	2.97±1.35	0.08±0.04	10.82±2.83	17.37±3.33[c]

[a] Cultures sampled 9 days after transfer to fresh medium with 0.3 mg per l 2,4-D.
[b] $t = 0.005$.
[c] $t = 0.05$.

TABLE 3

RELATIONSHIP BETWEEN EMBRYOGENESIS AND VOLATILE EMISSIONS IN *PHOENIX DACTYLIFERA* CALLUS CULTURES

Embryos/Culture	2,4-D	No. Cultures	Volatiles/g Fresh Weight			
			CO_2	C_2H_4	CH_3CHO	CH_3CH_2OH
	mg/l		%	*ppm*	*nmol*	*nmol*
0-1	0	3	2.83	0.11	168.84	974.94
5-16		3	4.25	0.63	49.65[a]	296.88[b]
0-1	3	3	3.96	0.21	150.36	1055.00
5-16		6	3.53	1.76	77.56[a]	416.28[b]
0-1	10	2	4.99	0.28	175.41	1292.16
5-16		3	3.23	0.59	117.10[b]	910.67[a]
0-1	30	5	1.62	0.25	193.28	1907.37
5-16		1	1.55	0.73	345.52	1006.13

[a] $t = 0.025$.
[b] $t = 0.010$.

introducing a stream of moist, filtered air, might enhance somatic embryogenesis in some callus cultures.

The available information on ethanol in plants indicates that this compound may be more than just a symptom of restricted aeration. In tissue cultures, endogenous ethanol is elevated by auxin and is associated with depressed levels of somatic embryogenesis; exogenous ethanol reversibly inhibits somatic embryogenesis (9). In a number of plant systems, ethanol has been reported to promote growth responses (15-19). Whereas both ethylene and ethanol emissions respond to auxin, the independent biosynthetic paths in plants of these compounds and their differences in physical properties, notably water solubility and volatility, would be consistent with independent roles as growth factors.

Changes in levels of ethanol, a typical product of anaerobic metabolism, may possibly reflect fundamental metabolic changes associated with the onset of organogenesis (14). Whether embryogenesis or organogenesis in vitro is accompanied by changes in the activity of enzymes, such as alcohol dehydrogenase, and whether endogenous ethanol serves per se as an inhibitor of organogenesis remain to be determined. The possibility that the 2,4-D effects are a result of increased ethylene is not supported by our data; ethylene emissions failed to show consistent enhancement in response to 2,4-D.

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