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DIFFERENTIATION RESPONSES IN CALLUS CULTURES OF *DATURA INNOXIA* BY PHOSPHOLIPID PRECURSORS

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Key Word Index—*Datura innoxia*; Solanaceae; callus; lipid precursors; choline; ethanolamine; greening; shoot differentiation; membrane lipids.

Abstract—The lipid composition of callus cultures of *Datura innoxia* grown on media supplemented with phospholipid precursors was studied. Choline or ethanolamine supplementation inhibited greening and shoot differentiation and led to an increase in the relative levels of the major phospholipids, phosphatidylcholine and phosphatidylethanolamine and to a decrease in the levels of galactolipids. The relative level of phosphatidylinositol which exhibited an inverse relationship with the degree of differentiation, was increased to a level comparable to that of the non-green undifferentiated callus.

INTRODUCTION

Plant membrane-lipids are involved in the regulation of membrane-bound enzymes such as plasma-membrane ATPase in radish [1] and microsomal NADH-cytochrome *c* reductase in potato tuber [2]. Several reports are available which suggest a regulatory role for membrane lipids in the developmental process [3]. A few studies have been undertaken to modify the lipid composition of plants, by exogenous supply of lipid precursors, and thus to elucidate its functional significance [4, 5]. Lipid precursors such as fatty acids in the form of esters and water-soluble precursors of phospholipid synthesis such as choline and ethanolamine were employed. Several reports demonstrated the effect of exogenously supplied fatty acids on physiological responses such as coleoptile growth in *Avena* [6], seed germination and seedling growth in cotton [7] and seed germination in rice [8]. Recently, studies on the modification of fatty acid composition by addition of fatty acids to cell cultures of soybean highlighted the suitability of such systems for studying the functional significance of lipids [9, 10].

In a previous study, we characterized the membrane-lipid composition of greening and shoot-differentiating callus cultures of *Datura innoxia* Mill [11]. The cultures showed a decrease in the relative levels of the major phospholipids (PC‡ and PE) due to both processes of differentiation. This paper describes the changes in the membrane-lipid composition of callus cultures brought about by supplementation of the cultures with the phospholipid precursors, choline and ethanolamine, and its subsequent effect on the differentiation responses.

RESULTS AND DISCUSSION

Lipid precursor supplementation and the differentiation responses

The greening and shoot differentiation responses of non-green callus cultures when grown in the appropriate growth media were inhibited, when the media were supplemented with either of the phospholipid precursors, ethanolamine or choline (Table 1). The dry-matter accumulation was reduced by some 13–18% whilst the chlorophyll content was reduced by *ca* 67–86%.

Lipid changes due to precursor supplementation

Addition of choline or ethanolamine to the auxin-containing medium led to a marginal increase (*ca* 18%) in the total lipid-content of the cultures without affecting the relative levels of the different lipid classes (Table 2). Supplementation of either of the cytokinin-containing media, which differed only in the concentration of kinetin, led to a decrease in the relative levels of galactolipids of *ca* 21–34%. There was also a decrease of between 18% (ethanolamine) and 17% (choline) in the total lipid content on supplementation of the high concentration cytokinin media with either choline or ethanolamine.

Among the different cultures supplemented with either of the precursors, only those grown on auxin-containing media showed an increase (17%, ethanolamine; 25%, choline) in the content of membrane-lipids (Table 3). The cultures grown on the precursor supplemented cytokinin media showed a decrease in the content of membrane lipids which was maximal (*ca* 35%) when choline was added to the high concentration cytokinin medium. A decrease in the contents of total lipids and membrane lipids and in the relative level of galactolipids due to precursor supplementation to cytokinin media marked

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‡Abbreviations: DGDG, digalactosyl diglyceride; MGDG, monogalactosyl diglyceride; NAA, naphthaleneacetic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SL, sulpholipid.

Table 1. Effect of choline and ethanolamine on the morphology, dry-matter accumulation and chlorophyll content of callus cultures of *D. innoxia*

Growth supplement*	Callus morphology	Dry-matter accumulation (mg/culture tube)	Total chlorophyll (mg/g fr. wt)
NAA (1 μM)	Non-green; undifferentiated	97	0.01
NAA (1 μM)+ choline	Non-green; undifferentiated	83	0.01
NAA (1 μM)+ ethanolamine	Non-green; undifferentiated	96	0.01
Kinetin (1 μM)	Green	120	0.13
Kinetin (1 μM)+choline	Pale green	101	0.03
Kinetin (1 μM)+ethanolamine	Pale green	105	0.04
Kinetin (10 μM)	Green; shoot differentiating; differentiation nodules present	131	0.29
Kinetin (10 μM)+choline	Pale green; no shoot differentiation	108	0.04
Kinetin (10 μM)+ethanolamine	Pale green; no shoot differentiation	113	0.04

*Added to the B-5 medium [21]; final concentration of choline/ethanolamine was 5 mM.

Table 2. Effect of choline and ethanolamine on the total lipid composition of callus cultures of *D. innoxia*

Growth supplement*	Total lipid (mg/g dry wt)	Amount (% total)		
		Phospho-lipids	Galacto-lipids	Neutral lipids
NAA (1 μM)	25.2	60	24	16
NAA (1 μM)+choline	30.2	66	21	13
NAA (1 μM)+ethanolamine	29.7	63	20	17
Kinetin (1 μM)	31.8	53	29	18
Kinetin (1 μM)+choline	28.9	58	23	19
Kinetin (1 μM)+ethanolamine	29.4	57	22	21
Kinetin (10 μM)	35.4	52	32	16
Kinetin (10 μM)+choline	26.0	57	21	22
Kinetin (10 μM)+ethanolamine	29.1	62	24	14

*Concentration of choline/ethanolamine was 5 mM.

Table 3. Effect of choline and ethanolamine on the phospholipid and galactolipid composition of callus cultures of *D. innoxia*

Growth supplement*	Phospholipids + galactolipids (μmol/g dry wt)	Phospholipids + galactolipids (% total)								
		PC	PE	PI	PS	PG	PA	MGDG	DGDG	SL
NAA (1 μM)	26.7	29	23	9	6	4	3	12	10	4
NAA (1 μM)+choline	33.4	39	17	9	5	3	4	11	9	3
NAA (1 μM)+ethanolamine	31.2	29	26	10	5	3	3	12	9	3
Kinetin (1 μM)	34.7	21	12	18	6	4	3	21	11	5
Kinetin (1 μM)+choline	28.0	30	19	12	6	4	3	12	10	4
Kinetin (1 μM)+ethanolamine	29.6	31	23	8	4	3	4	14	9	4
Kinetin (10 μM)	36.7	21	14	13	9	4	3	22	9	5
Kinetin (10 μM)+choline	24.4	27	18	11	8	4	4	14	10	4
Kinetin (10 μM)+ethanolamine	29.8	31	22	9	4	3	4	14	9	4

*Concentration of choline/ethanolamine was 5 mM.

the reversal of the changes which occurred during greening and shoot differentation in these cultures [11].

In the membrane-lipid composition, only those cultures grown on choline-supplemented auxin-containing medium showed specificity of accumulation in the respective phospholipids i.e. PC (Table 3). Choline supplementation led to an increase in the relative level of PC by *ca* 35% along with a decrease in the relative level of PE by *ca* 26%. By contrast, supplementation with either choline or ethanolamine to the cytokinin media led to an increase in the relative levels of both PC and PE. The increase in PC and PE due to choline supplementation was *ca* 43 and 58% respectively in the low-concentration cytokinin medium and that in the high-concentration cytokinin medium was *ca* 29% in both cases. Similarly, the increase in PC and PE due to ethanolamine supplementation was *ca* 48 and 92% respectively in the low concentration cytokinin medium and that in the high concentration cytokinin medium was *ca* 48 and 57% respectively.

Even though choline is known to have specificity to PC among membrane lipids [12], the synthesis of PC has been shown to occur through three pathways, i.e. via CDP-choline, trimethylation of PE or phosphorylethanolamine and a base exchange reaction with other lipids [13, 14]. The lack of specificity in the increase of PE on ethanolamine supplementation of the cytokinin media in cultures of *D. innoxia* (Table 3) may indicate its channelling into PC synthesis via the trimethylation pathway [13]. Induction of the trimethylation pathway due to cytokinin has also been reported in the axillary buds of tobacco [15].

In the present study, cultures of *D. innoxia* displayed uniformity in the cumulative level of PC and PE which was maintained within a range of *ca* 45–56% in different cultures grown on choline or ethanolamine supplemented media (Table 3). Moore and Troyer reported the regulation of the level of PC as a proportion of total phospholipids during the post-germination period in castor bean endosperm [16]. This report lends support to a comparable mode of regulation of the level of PC and PE as a proportion of total membrane lipids in the precursor supplemented cultures of *D. innoxia*.

Besides the changes in the major phospholipids, the level of PI in the cultures grown on precursor-supplemented cytokinin media was decreased to a level comparable to that of the non-green undifferentiated callus (Table 3). Precursor supplementation also led to a decrease in the relative level of MGDG and the ratio of MGDG/DGDG, the relvance of which is discussed elsewhere in relation to the greening and shoot differentiation responses in these cultures [11].

The results of the present study reveal a negative control of differentiation due to the exogenous supply of lipid precursors. It would be of interest to try to achieve a 'conditioning' of the cells to a specific developmental stage by means of modifying the lipid composition.

EXPERIMENTAL

Chemicals. Choline chloride and ethanolamine were from Sigma Chemical Company, St Louis, MO, USA.

Plant material and growth conditions. Non-green callus cultures of *D. innoxia*, initiated from leaf explants, were maintained on a semi-solid B-5 medium [21] with 10^{-6} M NAA. The non-green calli were transferred to B-5 medium with kinetin at 10^{-6} and 10^{-5} M to induce greening and shoot differentiation, respectively. These cultures were the controls to those grown on the lipid-precursor supplemented media. For the determination of biomass production, 200 mg fr. wt of callus was used as inoculum. Cultures were grown at 26° under white light from a fluorescent light source (intensity: 1200 $\mu W/cm^2$ at tissue level). Expts were carried out using 28-day-old callus cultures.

Precursor supplementation. Choline (as choline chloride) or ethanolamine was added to the different growth media to give a final concn of 5 mM. Stock solns of the precursors were neutralized using HCl.

Lipid analysis. This was carried out using a two step chromatographic procedure involving silica gel CC and TLC as reported elsewhere [17]. Determination of total lipids and dry-matter content was done gravimetrically and that of phospholipids and galactolipids was accomplished through lipid phosphorous [18] and lipid galactose [19], respectively. Chlorophyll content was estimated according to the procedure of Arnon [20].

Data presentation. Results are the mean of three expts. The standard deviations for different parameters are as follows: total lipids, neutral lipids and dry-matter content, *ca* 6%; total as well as individual phospholipids and galactolipids and total chlorophylls, ca. 3%.

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