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GREENING AND SHOOT-DIFFERENTIATION RELATED LIPID CHANGES IN CALLUS CULTURES OF *DATURA INNOXIA*

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

Abstract—Greening and shoot-differentiation related changes in lipid composition were studied in callus cultures of *Datura innoxia*. Both processes were associated with an increase in the content of the total lipids and the galactolipid fraction. There was also an increase in the relative level of chloroplast specific monogalactosyl diglyceride. The membrane lipid composition of tissues at different degrees of differentiation revealed an apparently inverse relationship between the degree of differentiation and the absolute as well as the relative level of phosphatidylinositol. The results are discussed with respect to the relevance of changes in membrane lipid composition during differentiation.

INTRODUCTION

Tissues in culture undergoing shoot differentiation or plantlet regeneration undergo an initial phase of greening [1, 2]. Subsequently, the green callus forms morphologically distinct shoot primordia or 'nodules' which develop into shoots [3, 4]. Thus, the differentiation process is found to be specifically localized in the system [5, 6]. Several studies have been undertaken to sequence the developmental events in order to understand the process of differentiation [2, 4, 7–9]. Establishment of physiological gradients by the accumulation of carbohydrates [7], malate [8] and an energy pool in the form of ATP, ADP, NADH and NADPH [9] are found to occur during the formation of the nodules. The above parameters are correlated to the increased respiratory activity of the nodules during differentiation.

Studies have also been reported on the ultrastructural and biochemical changes associated with greening [10, 11]. These studies, involving both intact plants [12, 13] and tissues in culture [14, 15], have shown that major changes occur in the membrane lipids of chloroplasts during greening. Recently, some direct evidence has been presented for the role of plant membrane lipids in the regulation of membrane-bound enzymes [16, 17]. In particular, the major fraction of the extrachloroplast lipids, i.e. phospholipids, is found to regulate these membrane-bound enzymes [17]. In spite of the functional significance of membrane lipids, there has been no study made to characterize differentiating systems on the basis of membrane lipid composition.

This paper describes the changes in the membrane lipid composition in greening and shoot-differentiating callus cultures of *Datura innoxia* Mill.

RESULTS

Lipid changes during greening and shoot differentiation

The non-green callus underwent the processes of greening and shoot differentiation when transferred to the appropriate growth media. The chlorophyll content of both the green and shoot-differentiating callus tissues increased by *ca* 13- and 29-fold, respectively, as compared to the control non-green callus (Table 1). Whereas the green callus was uniformly green, the shoot-differentiating callus developed morphologically distinct 'nodules' which subsequently developed into shoots. The chlorophyll content of the green and nodular regions of the shoot-differentiating callus showed an increase of *ca* 17- and 92-fold, respectively, as compared to the non-green callus.

Greening and shoot differentiation were associated with an increase in the content of total lipids by *ca* 26 and 40%, respectively (Table 2). This increase was reflected in the relative level of galactolipids, which showed an increase of *ca* 21 and 33% in the green and shoot-differentiating callus tissues, respectively. There was only a marginal decrease in the relative level of phospholipids in both processes. Greening and shoot differentiation

Table 1. Chlorophyll content of different callus tissues of *D. innoxia*

Tissue	Total chlorophyll (mg/g fr. wt)
Non-green undifferentiated callus	0.01
Green callus	0.13
Total shoot-differentiating callus	0.29
Green region of shoot-differentiating callus	0.17
Nodular region of shoot-differentiating callus	0.92

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Table 2. Greening and shoot-differentiation related changes in the total lipid composition of callus cultures of *D. innoxia*

Tissue	Total lipid (mg/g dry wt)	Amount (% total)		
		Phospho-lipids	Galacto-lipids	Neutral lipids
Non-green undifferentiated callus	25.2	60	24	16
Green callus	31.8	53	29	18
Total shoot-differentiating callus	35.4	52	32	16
Green region of shoot-differentiating callus	35.0	53	30	17
Nodular region of shoot-differentiating callus	41.0	55	31	14

were not associated with any significant change in the relative level of neutral lipids.

Both the processes of differentiation (greening and shoot differentiation) were associated with an increase in the content of membrane lipids by *ca* 30 and 37%, respectively (Table 3). However, the differentiation process was not associated with any qualitative change in the membrane lipid composition, as PC*, PE, PI, PS, PG, MGDG, DGDG and SL were all present in the non-green, green and shoot-differentiating callus cultures. Whereas there was a decrease in the relative level of major phospholipids (PC and PE), PI showed a marked increase in both the green and shoot-differentiating callus which accounted for *ca* 100 and 44%, respectively, as compared to the non-green callus. The decrease in PC was *ca* 28% in both cases and that of PE was *ca* 48 and 39% in the green and shoot-differentiating callus tissues, respectively. Whereas the relative level of PS was unchanged in the green callus, shoot-differentiating callus showed an increase of *ca* 50%. The relative level of the other minor phospholipids, PG and PA, did not undergo any change due to either of the differentiation responses.

Among galactolipids, changes were marked by an increase in the relative level of the major chloroplast lipid, MGDG, in both the green and shoot-differentiating callus tissues, to comparable degrees (Table 3). The level of DGDG was maintained constant. A subsequent increase in the relative level of MGDG led to an increase in the ratio of MGDG/DGDG to 1.9 and 2.4 in the green and shoot-differentiating callus tissues respectively, as against 1.2 in the non-green callus.

Zonal differences in the lipid composition of shoot-differentiating callus

Since the shoot-differentiating callus exhibited two morphologically distinct regions, i.e. green and nodular regions, studies were undertaken to compare the lipid composition between the zones of the callus.

The nodules (3 nodules/4.5 g fr. wt of the total shoot-differentiating callus/culture tube) formed *ca* 18% of the differentiating callus on a fresh weight basis. The content of the total lipids (Table 2) and the total membrane lipids (Table 3) of the nodules were higher by *ca* 17% as compared to the green region. Whereas there was no difference in the relative levels of phospholipids, galactolipids and neutral lipids between the green and nodular regions (Table 2), a difference was observed in the relative level of one of the membrane lipids, i.e. PI (Table 3). The level of PI was lower in the nodules by *ca* 43% as compared to the green region. Among the different galactolipids, no significant difference was observed between the zones of the shoot-differentiating callus.

PI content and degree of differentiation

With the recently accumulating evidence for the increased turnover rate of PI lipids and their cleavage products (inositol phosphates), acting as second messengers [18], the PI content of the different tissues of *D. innoxia* was measured.

On the basis of morphology and chlorophyll content (Table 1), the different tissues employed in the present study can be arranged in decreasing order of their degree of differentiation (Table 4). The leaves with a total chlorophyll content of 1.8 mg/g fr. wt and total membrane lipid content of 83 μmol/g dry wt [19] represent the highest degree of differentiation. The absolute as well as the relative level of PI of the different tissues showed an inverse relationship with the degree of differentiation, barring the odd position of the non-green undifferentiated callus (Table 4).

DISCUSSION

Greening involves the net synthesis of the grana membrane with an associated increase in the chloroplast-specific galactolipids [11]. Among the different galactolipids, MGDG and DGDG are specifically localized in the grana and envelope membranes of the chloroplasts, respectively [13]. This specificity in the distribution of membrane lipids provides a marker for evaluating the developmental stage of chloroplasts [13]. Accordingly, mature chloroplasts with fully developed grana have a higher ratio of MGDG/DGDG in contrast to the poorly developed chloroplasts and proplastids. Studies employing cultures of tobacco [20] and *Ricinus* sp. [15] showed the occurrence of such typical changes in chloroplast-specific lipids during greening. These changes were also observed in the present study (Tables 2 and 3).

The changes in membrane lipid composition during developmental processes are of possible functional signifi-

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

Table 3. Greening and shoot-differentiation related changes in the phospholipid and galactolipid composition of callus cultures of *D. innoxia*

Tissue	Phospholipids + galactolipids (μmol/g dry wt)	Phospholipids and galactolipids (% total)								
		PC	PE	PI	PS	PG	PA	MGDG	DGDG	SL
Non-green undifferentiated callus	26.7	29	23	9	6	4	3	12	10	4
Green callus	34.7	21	12	18	6	4	3	21	11	5
Total shoot-differentiating callus	36.7	21	14	13	9	4	3	22	9	5
Green region of shoot-differentiating callus	36.0	20	14	14	8	4	3	22	10	6
Nodular region of shoot-differentiating callus	42.4	23	16	8	7	5	3	23	9	6

Table 4. Phosphatidylinositol content of tissues at different degrees of differentiation in *D. innoxia*

Tissue*	PI content	
	(μmol/g dry wt)	(% phospholipids + galactolipids)
Leaves	3.3	4
Nodular region of shoot-differentiating callus	3.4	8
Total shoot-differentiating callus	4.8	13
Green region of shoot-differentiating callus	5.0	14
Green callus	6.2	18
Non-green undifferentiated callus	2.4	9

*Arranged in order of decreasing degree of differentiation.

cance [21]. Studies employing model systems, such as liposomes and reconstituted lipoprotein vesicles, revealed the role of membrane lipids in the regulation of membrane phenomena such as the activity of membrane-bound enzymes and hormonal receptors [22, 23]. A few studies with plant systems have also established the regulatory role of membrane lipids [16, 17].

In the present study, comparison of the membrane lipid composition of tissues at different stages of differentiation revealed significant differences in the level of PI (Table 4). In recent years, mostly from studies with animal systems, there has been ample evidence for the role of PI in stimulant-induced responses [18]. Stimulants, such as acetylcholine and epidermal growth factor, act by bringing about a change in the turnover rate of PI. Different phosphorylated forms of PI and their lipolytic products, i.e. inositol phosphates and diglyceride, constitute the PI cycle in the stimulated tissues. Recently, a few studies have shown the presence of different components of the PI cycle in plant tissues [24, 25].

The observations in the present study as to the differences in the membrane lipid composition among different tissues, especially that in the level of PI, could indicate the possible involvement of membrane lipids in the process of differentiation.

EXPERIMENTAL

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 [26] supplemented with 10^{-6} M naphthaleneacetic acid. The non-green calli were transferred to B_5 medium supplemented with kinetin at 10^{-6} and 10^{-5} M to induce greening and shoot differentiation, respectively [19]. Cultures were maintained by subsequent transfers to the appropriate nutrient media after every 30 days. Cultures were grown at 26° under white light from a fluorescent light source (intensity 1200 $\mu W/cm^2$ at tissue level). Experiments were carried out using 28-day-old callus tissues. Young green leaves were obtained from nursery-grown plants.

Lipid analysis. This was carried out using a two-step chromatographic procedure involving silica gel CC and TLC as reported elsewhere [12]. Determination of total lipids and dry matter content was done gravimetrically and that of phospholipids and galactolipids was accomplished through lipid phosphorus [27] and lipid galactose [28], respectively. Chlorophyll content was estimated according to the procedure of Arnon [29].

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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