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CHANGES IN LIPID COMPOSITION DURING FLORAL DEVELOPMENT OF *BRASSICA CAMPESTRIS*

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Key Word Index—*Brassica campestris*; Cruciferae; sterol biosynthesis; floral development; phospholipids; cholesterol; cerebrosides.

Abstract—Apical tissues of *Brassica campestris*, grown under controlled environmental conditions, were analysed for their lipid content. The principal lipids were sterols, phospholipids and sphingolipids. The major sterols were identified as sitosterol, stigmasterol, campesterol and cholesterol, the phospholipids as phosphatidylethanolamine (PE) and phosphatidylcholine (PC), and the sphingolipids as cerebrosides. In the early stages of apical development, unusually high proportions of cholesterol and cerebrosides were found. However, their relative proportions gradually decreased as the apex developed; a concomitant increase in sitosterol was observed. These results suggested a specific association between these lipids and the development of the shoot apex. PE increased steadily during apical development, whereas PC increased more rapidly, but then declined at the later stage. The relative proportion of campesterol increased in the apex during the late stages of development and appeared to be involved in petal formation, which coincided with the decrease in PC.

INTRODUCTION

In addition to their widely recognized roles as architectural components of cell membranes, phospholipids and sterols are now seen to have regulatory influences over a variety of metabolic events [1–3]. It is likely that these influences are the consequence of development, and studies have suggested that changes in phospholipid content are a response to physiological processes, such as fruit ripening [4], senescence [5, 6] and ageing [7]. However, trace amounts of ergosterol (in yeasts) and stigmasterol (in higher plants) are essential to 'trigger' cell division [8–10], and the production of the metabolically important phospholipids, phosphatidylinositol (PI) and phosphatidylcholine (PC), may be regulated by the sterol content and/or composition of cellular membranes [11–14].

Cellular sterol content and composition may also play a regulatory role in important physiological events in the life-cycle of higher plants. For example, previous research with monocotyledonous (sorghum) and dicotyledonous species (soybean, squash) revealed that up to the time of floral differentiation, total amounts of free sterol in the whole plant increased, but during inflorescence development, sterol synthesis either appeared to decline or remained static [15, 16]. It was suggested that this indicated reduced synthesis or a rapid turnover (or both) of sterols at, or just prior to, flowering. Analysis of the sterol content and composition of developing floral apices from *Lolium temulentum* confirmed that the sterol content declined during the inductive period and that a much greater amount of cholesterol was present in the apex, compared with other parts of the plant [17]. It was suggested that such changes in sterol composition mediated membrane permeability, and that this, in turn, might affect the transport of metabolites during evocation.

In the work presented here, the changes in sterol and phospholipid composition were examined in the apex of a dicotyledonous plant (*Brassica campestris*) during floral development.

RESULTS AND DISCUSSION

Apical development in *B. campestris* was studied and scored to determine the morphological changes occurring with respect to the age of the plant (Table 1). The classification provides a simple guide to apical development and follows an orderly pattern comparable to that of other brassicas [18, 19]. However, for a more precise study, removal of the sepals is essential to identify and classify the developing floral organs. For this work, such intricate dissections were considered neither

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Table 1. Description and numerical value for stages of apical development in *Brassica campestris*

Stage	Age (days)	Description
0	2	*Germinating seedling.* Flat apical meristem, first true leaves forming.
1	2–6	*Vegetative stage.* Flat apical meristem, surrounded by leaf primordia.
2	7	*Floral induction.* Inflorescence apices beginning to develop at the base of the meristem. The meristem becomes more convexed.
3	8–10	*Early sepal development.* Sepals apparent as broad ridges at the base of the formed floral apices.
4	10–12	*Sepal development.* Sepals continue to develop over the formed floral apices which are constricting at the base. The younger floral apices begin to develop sepals
5	11–12	*Late sepal development.* Sepals hook over the older floral apices, protecting the development of other floral organs.
6	11–13	*Early peduncle development.* Apices covered by the sepals, the peduncle begins to elongate.
7	13–15	*Late peduncle development.* Stems continue to elongate.
8	15–18	*Apex emergence.* Well formed buds begin to emerge from the top of the shoot apex.
9	18	*Bud break.* Older floral buds are opening and younger buds are well developed.

practical nor necessary. The apices remained morphologically unaltered until day 7 when transition to reproductive development occurred. This was followed by sepal development, swelling of the flower buds, as they reached maturity, and elongation of the peduncles. Flower bud break occurred after day 18.

Sterol composition for developing shoot apices was determined each day from day 6 (GS-1), prior to evocation, to day 18 (GS-9). In apical tissue, the major free sterols were sitosterol, cholesterol and campesterol, with stigmasterol present in trace amounts (Table 2). However, in other organs, all four sterols were found, although cholesterol was only a minor component (data not shown). The most marked changes in the amounts of sterol were seen between cholesterol and sitosterol. Initially, cholesterol was most abundant, 66.2% (±2.4) (between GS-1 and -2) of total sterol, with sitosterol at 19.2% (±2.4). During the transition to flowering, the relative amount of cholesterol decreased, whilst that of sitosterol increased, so by GS-9, cholesterol represented 11.6% (±0.9) of the total sterol and sitosterol 66.9% (±1.4) (Table 2; Fig. 1). Statistical analysis (linear regression) showed a significant inverse correlation (correlation coefficient = −0.990, $p = 0.1\%$) between these two sterols at each stage of development. Similar results were reported in *L. temulentum* [17], *Hordeum vulgare* and *Xanthium strumarium* [20]. These findings show an important role for cholesterol in plant development and the significance of the inverse correlation suggests that sitosterol may be regulating the production of cholesterol, or *vice versa*, by feed-back inhibition at the cycloartenol methylation stage.

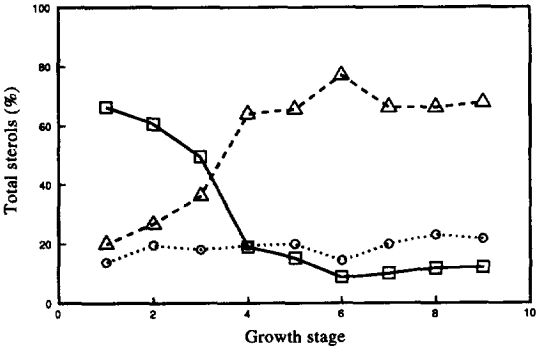

Fig. 1. Change in cholesterol (□), sitosterol (△) and campesterol (○) composition (as % total sterols) in shoot apex of *Brassica campestris* during floral development. (LSD = 12.9, $n = 27$.)

Prior to GS-8, campesterol accounted for 14% of the total sterol content. However, just before bud break, the percentage campesterol increased to 21.5% (Table 2). This coincided with petal development. An earlier study of sterol distribution in different plant organs (data now shown) showed campesterol to be the dominant sterol in petals. In plants treated with the experimental herbicide, γ-ketotriazole, which inhibits sterol biosynthesis, petal formation was suppressed [21]. This may have been caused by the inhibition of campesterol biosynthesis by the herbicide, and the observation raises the possibility that campesterol may be essential for petal formation. However, further work is required to confirm this.

At floral induction the major lipids present were the

Table 2. Relative sterol composition (%) of the apex of *Brassica campestris* during floral initiation and development. Values represent the mean of three (GS-1 and -3) or five (GS-6 and -9) replicates (±SE). Stigmasterol was present only in trace amounts

	Growth stage			
	1	3	6	9
Cholesterol	66.2±2.4	47.7±1.3	8.5±1.7	11.6±0.9
Sitosterol	19.2±2.4	35.4±0.9	77.2±1.8	66.9±1.4
Campesterol	13.6±1.1	16.9±1.1	14.3±2.6	21.5±0.6

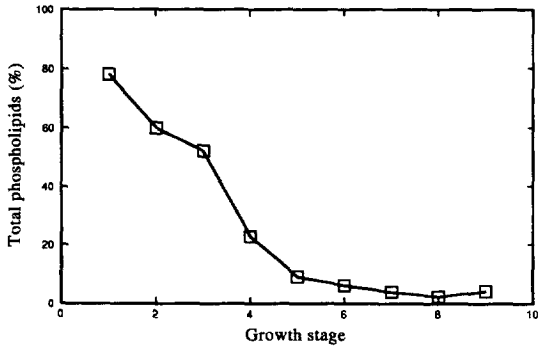

Fig. 2. Change in cerebroside composition (as % total phospholipids, galactolipids and sphingolipids) in shoot apex of *Brassica campestris* during floral development.

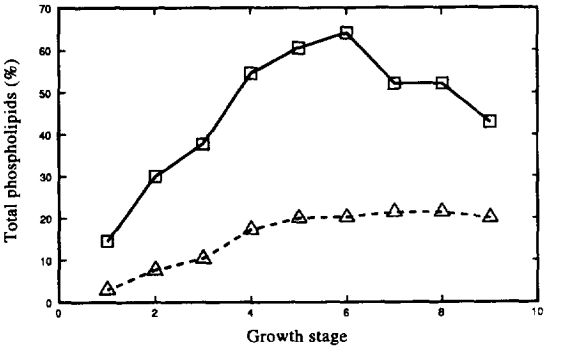

Fig. 3. Change in PE (△) and PC (□) composition (as % total phospholipids, galactolipids and sphingolipids) in shoot apex of *Brassica campestris* during floral development. (LSD = 13.4, $n = 18$.)

cerebrosides, representing almost 80% of the total phospholipids and sphingolipids detected. However, the proportion declined rapidly until GS-5, when they accounted for less than 10% of the lipid fraction (Fig. 2). Regression analysis showed a significant correlation between the relative proportions of cholesterol and cerebrosides (correlation coefficient = 0.988, $p = 0.1\%$), implying coordinate regulation of synthesis. Indeed, van Blitterswijk *et al.* have shown that cholesterol partitions preferentially into membranes with a high sphingolipid content [22]. It is also thought that, in plant plasma membranes, cerebrosides play a role in cryostability [23] and the acclimation of plants to moderate water-stress [24]. Cholesterol is also known to stabilize membranes [25]; it is possible that the lipid composition of the apex during floral induction is geared to providing maximum membrane stability and protection against environmental fluctuations.

In the floral apex, PI, phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) were only minor lipid components (Table 3) and changes observed may be attributed to membrane expansion during cell development. The galactolipid, monogalactosyldiacylglycerol (MGDG), constituted 4.65% of the lipid fraction at GS-6. However, by GS-9 it represented 16.3% (Table 3). Galactolipids are always associated with chloroplasts or non-photosynthetic plastids. Therefore, the increase will be associated with the development of photosynthetic tissue, such as sepals and peduncles.

PC and phosphatidylethanolamine (PE) showed significant changes during the growth and development of the apex (Fig. 3). The PE content increased from GS-1 (4%) to GS-6 (>20%) after which it remained constant. Over the same period, PC increased from 15 to 63%, but declined to 42.9% at GS-9 (Fig. 3). Consequently, there was an increase in the PE:PC ratio from 0.26 to >0.3, corresponding to the decrease in PC at the later growth stages (Fig. 4). Linear regression showed that there was an inverse correlation between the relative proportions of PC and cholesterol (correlation coefficient = −0.895, $p = 0.1\%$). This is contrary to that in cultured myogenic cells, where a reduction in cholesterol content, brought about by the addition of 25-hydroxycholesterol or compactin, decreased cholinephosphate cytidyltransferase activity, the rate

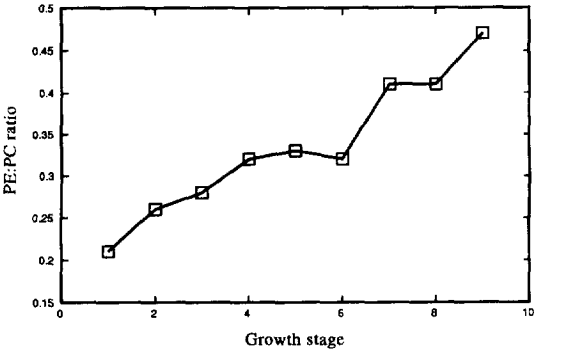

Fig. 4. Change in PE:PC ratio in shoot apex of *Brassica campestris* during floral development.

Table 3. Relative composition (%) of minor phospholipids and galactolipids in the apex of *Brassica campestris* during floral initiation and development. Values represent the mean of three (GS-1 and -3) or five (GS-6 and -9) replicates (±SE)

	Growth stage			
	1	3	6	9
MGDG	1.28±1.03	0.05±0.04	4.65±0.62	16.3 ±0.90
PG	0.23±0.10	nd*	0.46±0.30	4.00±0.82
DPG	0.24±0.13	0.03±0.02	3.51±0.41	7.00±1.10
PI	0.41±0.15	nd*	1.84±0.84	4.73±0.63

*Not detected.

limiting enzyme of PC synthesis [7]. However, there was a positive correlation between PC and sitosterol (correlation coefficient = 0.923, $p = 0.1\%$), suggesting, in plants, that sitosterol is important in the regulation of PC synthesis, implying coordinate regulation of lipid synthesis. It was also interesting to note that the decline in PC (Fig. 3) coincided with the increase in campesterol and petal formation, but it is not possible to say whether these events are connected.

In conclusion, the developing floral apex represents a highly active system in which sterol and phospholipid/sphingolipid composition change rapidly. It is possible that these changes might be involved in regulating the transition from the vegetative to the flowering state of the apex or, perhaps, more likely, conferring maximum stability and protection to it at this critical stage of development.

EXPERIMENTAL

Plant growth. Seeds of *B. campestris* (cv. Wisconsin Fast) were grown in compost in a controlled environment, 16 hr day, 8 hr night at 20°, relative humidity 70%, with photosynthetically active radiation of 350 μmol m^{-2} sec^{-1}. 20 plants were collected daily from 6 to 18 days after sowing. Plants were selected for uniformity of height and expanded cotyledons. Each assay was replicated three times and data analysed using unpaired '*t*' tests.

Lipid extraction and analytical techniques. This was done using the modified method described in ref. [26]. Apical tissue (2.5–5 mg fr.wt) was weighed accurately into glass vials and 0.5 ml MeOH added. After the addition of 15 μg L-3-phosphatidyl-*N,N*-dimethylethanolamine and 5 μg β-cholestanol as int. standards, the mixt. was heated at 60° for 20 min. $CHCl_3$–MeOH (1:2) (0.75 ml) was then added, the mixt. shaken, a further 0.5 ml $CHCl_3$ added and the mixt. shaken again. H_2O (0.5 ml) was added to assist the sepn of the MeOH and $CHCl_3$ layers, which was completed by leaving the mixt. to stand at 4° for 10 min. The $CHCl_3$ layer was removed, evapd under N_2 and the residue dissolved in 50 μl $CHCl_3$. For sterol analysis, 20 μl of the $CHCl_3$ extract was taken, the solvent evapd under N_2 and the residue acetylated for at least 2 hr with 200 μl Ac_2O–pyridine (1:1). The solvents were evapd under N_2 and the residue redissolved in 10 μl EtOAc.

Phospholipids were analysed using an Econosphere Silica 3 μm, 150 × 4.6 mm HPLC column. Detection was by an evaporative light-scattering detector at 50° in a stream of N_2 (1.5 bar). The method was modified from that of ref. [27] and used a 3-solvent gradient system of (A) hexane–dimethoxypropane (99:1), (B) *iso*PrOH–$CHCl_3$ (4:1) and (C) *iso*PrOH–H_2O (1:1), at a flow rate of 2 ml min^{-1} throughout. The solvent programme was as follows: 100% A at time 0, and 1 min. At 5 min, A was 80% and B 20%. A, B and C were 42, 52 and 6%, respectively, at 5.1 min and at 15 min they were 35, 49 and 16%. At 20 min A was 42%, B 52% and C 6% and at 25 min A was 30% and B 70%. Finally, A was 100% at 30 min. Individual lipid classes were identified by co-chromatography with authentic standards.

Sterol acetates were analysed by capillary GC as described in ref. [26]. The identity of resolved sterol acetates was confirmed by comparison with authentic compounds and GC-MS.

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