Lipid composition of *Silybum marianum* cell cultures treated with methyl jasmonate

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Abstract

Elicitation of cell cultures of *Silybum marianum* with methyl jasmonate (MeJA) increases the production and release of the secondary metabolite silymarin into the culture medium and this process seems to be dependent on phospholipase D activity and its product phosphatidic acid (PA). However, MeJA did not alter total membrane lipid content or overall fatty acid composition. A progressive increase in some galactolipids was observed with elicitation time. Phospholipids were mainly represented by phosphatidylcholine (PC) followed by phosphatidylethanolamine (PE) and phosphatidylinositol (PI). MeJA caused losses of PC species that contain two unsaturated acyl species, 36:5 and 36:6 and an increase in 36:2 species. A drop in the ratio of compounds with 18:3 in PI and PE was also observed. The presence of the lysophospholipids (LP) LPC (16:0, 18:3, 18:2, 18:1) and LPE (16:0, 18:3, 18:2, 18:1) and the high contents of PA, represented by the molecular species 34:3, 34:2 and 36:5 and 36:4, indicates high basal level of phospholipase activity in cultures and a high phospholipid turnover. MeJA treatment did not quantitatively alter these lipid classes.

Additional key words: galactolipids, lipid signalling, lysophospholipids, phospholipids, secondary metabolites, silymarin.

Introduction

Silymarin is a constitutive natural compound of the fruits of *Silybum marianum* that is used for the treatment of liver diseases (Flora *et al*. 1998). It is well documented that plants can be stimulated by elicitors, resulting in increased accumulation of a variety of secondary metabolites (Repka *et al.* 2004, Gutierrez-Carbajal *et al*. 2010, Corrado *et al.* 2011). The strategy of elicitation has been also applied to *S. marianum* cell cultures and it has been demonstrated that treatment of cell suspensions with methyl jasmonate (MeJA) improved silymarin production and its release into the culture medium (Sánchez Sampedro *et al*. 2005a).

Jasmonates (JAs) have been shown to regulate secretion of nectar (Rhadika *et al*. 2010), secondary metabolites (Kim *et al*., 2006, Ruiz-May *et al*. 2009) or polysaccharides (Capataz-Tafur *et al*. 2010). As plant cell membranes are highly vulnerable to stress (Larkindale and Huang 2004, Luo *et al.* 2010), some authors explain the elicitor-induced excretion of secondary metabolites as consequence of disturbances of cell permeability. However, there is still insufficient knowledge of how JAs or MeJA induce the release of secondary metabolites. In *Arabidopsis,* phospholipases were up regulated by MeJA treatment and it has been proposed that the activity of these enzymes results in the modification of lipid constituents of the membrane and generation of one or more products that are able to recruit or modulate specific target proteins (Meijer and Munnik 2003). In *Brassica napus* leaves, phospholipase D (PLD) was rapidly activated upon MeJA treatment (Profotova *et al*. 2005). In sorghum, MeJA induced several putative phospholipases (Salzman *et al*. 2005). Recently, an increase of PLD activity after MeJA treatment has also been demonstrated in *S. marianum* cultures and formation of its product phosphatidic acid (PA), which acts as a second messenger under various biotic and abiotic stresses (Munnik 2001, Testerink and Munnik 2005) seems to be necessary for the formation and/or release of

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Abbreviations: BHT - butylated hydroxytoluene; DGDG - digalactosyldiacylglycerol; JA - jasmonate; LP - lysophospholipids; MeJA - methyl jasmonate; MGDG - monogalactosyldiacylglycerol; PA - phosphatidic acid; PC - phosphatidylcholine; PE - phosphatidylethanolamine; PG - phosphatidylglycerol; PI - phosphatidylinositol; PLD - phospholipase D; PS - phosphatidylserine.
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silymarin into the culture medium (Madrid and Corchete 2010). Yamaguchi *et al*. (2003) suggested that the elicitor-induced phytoalexins in rice suspension cell cultures may be mediated by PLD because the inhibition of PLD by *n*-butanol almost completely suppressed the elicitor-induced accumulation of momilactone A, a diterpenoid phytoalexin. It has also been proposed that the activation of PLD and the production of PA might act as a positive step in regulating taxol biosynthesis in *Taxus chinensis* cells (Yang *et al.* 2007).

Lipidomic strategies and biochemical methods are convenient tools to evaluate lipid metabolic pathways and specific enzymes involved in elicitation. This may help to improve the secondary metabolite production in induced cultures by manipulating post synthetic events like secretion. With this aim, in the present work a quantitative mass-spectrometry-based lipid profiling was employed to identify alterations in neutral lipid metabolism in cell cultures of *S. marianum* during the progression of elicitation with MeJA.

Materials and methods

The cell line used was established from *Silybum marianum* (L.) Gaertn. hypocotyl-derived callus. The growth medium was the same as that described in Sanchez-Sampedro *et al.* (2005b). Cultures were shaken at 90 rpm and subcultured every 2 weeks. Elicitation was done with 100 μM MeJA, prepared as a stock solution in ethanol (concentration of ethanol in cultures was 0.05 %) 3 d after subculture. Controls received equivalent volumes of solvents.

Total lipid extraction of *S. marianum* cells was performed following the method of Welti *et al*. (2002). In short, fresh cells (approximate 0.6 g) were immediately placed in 3 cm^3 isopropanol with 0.01 % butylated hydroxytoluene (BHT) at 75 °C. The tubes were incubated at 75 °C for 15 min, and 1.5 cm^3 chloroform and 0.6 cm^3 ultrapure water were added. The tubes were shaken for 1 h followed by removal of the extract. The cells were re-extracted with chloroform/methanol (2:1) containing 0.01 % BHT five times with 30 min of agitation each time. The combined extracts were washed once with 1 cm^3 1 M KCl and once with 2 cm^3 ultrapure water. The solvent was then evaporated under nitrogen and the resulting lipid samples were stored at -20 °C. The remaining cells were heated overnight at 105 °C and weighed. Prior to analysis, the extracted lipid samples were redissolved in 2 cm^3 chloroform/methanol (1:1, v/v). Samples from control and elicited cell cultures at each time period were analysed on a triple quadrupole tandem mass spectrometer (*4000 Q-TRA, MDS Sciex, Applied Biosystems*, Ontario, Canada) at Kansas Lipidomic Center (USA).

The fatty acid composition of cells was also determined by heating lipid extracts at 80 ºC in 1 cm^3 of 2.5 % (v/v) H_2SO_4 in methanol for 30 min in screw capped tubes; the saturated fatty acid heptadecanoic acid (17:0) was included as internal standard. After the addition of 1.5 cm^3 of water and 0.3 cm^3 of hexane, fatty acid methyl esters were extracted by centrifugation at low speed. Samples of the organic phase were separated and analysed by gas chromatography - mass spectrometry (GC-MS; *Kupé 5000*, *Shimidzu*, Japan) with a *SPB1* column (height 12 m, i.d. 0.2 mm and film thickness 0.3 μm) and quantified by electronic impact MS (70 eV). Typically 0.002 cm^3 were injected and chromatograms started at a column temperature of 100 ºC, which was raised up to a final temperature of 300 ºC after 10 min.

Results and discussion

Lipid extracts of *Silybum marianum* cells in suspension cultures contained mainly palmitic acid and linolenic acid. Fatty acid proportions were not significantly altered by elicitation with MeJA (Table 1).

Table 1. Fatty acid composition [%] of total lipids from cells of *Silybum marianum* suspension cultures 24 h after elicitation with 100 μM MeJA. Means ± SD, $n = 5$.

Fatty acid	Control	MeJA
14:00	10.0 ± 2.8	8.2 ± 3.1
16:02	1.0 ± 0.1	0.8 ± 0.2
16:01	8.7 ± 4.5	6.3 ± 1.2
16.00	38.3 ± 12.6	35.1 ± 14.5
18:03	18.3 ± 2.9	19.3 ± 3.9
18:02	6.5 ± 1.7	10.4 ± 3.4
18:01	9.0 ± 3.7	8.7 ± 2.1
18:00	8.1 ± 2.1	8.7 ± 1.9

Lipid profiling was applied to analyze changes in lipid molecular species of *S. marianum* cultures during the course of elicitation. MeJA treatment did not significantly alter the membrane lipid content over the 48 h studied period. Galactolipids were represented by monogalactosyldiacylglycerol (MDGG) and digalactosyldiacylglycerol (DGDG) species that contained polyunsaturated acyl species (36:6 and 36:5). The galactolipid contents were kept constant during elicitation, however, a progressive increase in 36:6 species and a decrease in 36:5 was observed with elicitation time (Tables 2,3).

Phosphatidylcholine (PC) was the most abundant phospholipid in cultures with predominantly unsaturated acyl species 36:5 and 36:6, followed by unsaturated species 34:3 and 34:2. Phosphatidylethanolamine (PE) showed a similar composition as PC while phospha-

Table 2. Total lipids, galactolipids and glycerophospholipids [nmol mg^{-1}(d.m.)] in *Silybum marianum* cells treated with 100 μM MeJA for 8, 24 and 48 h. Means ± SD, $n = 5$.

Compound	Control 8	MeJA 8	Control 24	MeJA 24	Control 48	MeJA 48
Total lipids	61.28 ±16.35	59.38 ±10.47	76.48 ±10.07	64.76 ± 7.04	57.79 ± 13.93	63.77 ± 8.27
Total DGDG	3.36 ± 1.24	2.95 ± 0.68	3.73 ± 0.48	4.09 ± 0.97	4.22 ± 1.55	4.19 ± 0.45
Total MGDG	3.66 ± 1.26	3.34 ± 0.72	4.23 ± 0.54	5.06 ± 0.49	4.51 ± 1.04	4.97 ± 0.51
Total PC	31.02 ± 7.87	25.96 ± 4.20	33.25 ± 4.78	27.30 ± 2.78	26.74 ± 6.02	26.46 ± 4.49
Total PE	9.45 ± 3.01	13.79 ± 2.43	18.51 ± 2.56	12.92 ± 2.61	9.50 ± 2.58	13.84 ± 1.98
Total PI	10.24 ± 3.19	10.34 ± 2.09	12.85 ± 1.55	11.99 ± 1.18	10.01 ± 2.30	10.88 ± 1.42
Total PG	1.24 ± 0.42	0.87 ± 0.15	1.27 ± 0.17	1.03 ± 0.11	0.93 ± 0.21	0.93 ± 0.13
Total PS	0.56 ± 0.19	0.78 ± 0.14	0.89 ± 0.12	0.80 ± 0.16	0.63 ± 0.19	0.78 ± 0.09

Table 3. Individual molecular species of galactolipids [nmol mg^{-1}(d.m.)] in *Silybum marianum* cells treated with 100 μM MeJA for 8, 24 and 48 h. Means ± SD, $n = 5$.

Compound	Control 8	MeJA8	Control 24	MeJA24	Control 48	MeJA48
DGDG (34:3)	0.23 ± 0.08	0.29 ± 0.07	0.28 ± 0.04	0.51 ± 0.07	0.31 ± 0.11	0.52 ± 0.05
DGDG (34:2)	0.16 ± 0.05	0.11 ± 0.03	0.19 ± 0.03	0.09 ± 0.01	0.31 ± 0.11	0.07 ± 0.01
DGDG (34:1)	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
DGDG (36:6)	1.75 ± 0.66	1.73 ± 0.41	1.97 ± 0.23	2.72 ± 0.69	1.68 ± 0.63	2.80 ± 0.32
DGDG (36:5)	0.84 ± 0.31	0.49 ± 0.11	0.97 ± 0.18	0.34 ± 0.09	1.34 ± 0.48	0.29 ± 0.03
DGDG (36:4)	0.21 ± 0.07	0.18 ± 0.05	0.24 ± 0.03	0.23 ± 0.07	0.37 ± 0.15	0.28 ± 0.04
DGDG (36:3)	0.09 ± 0.03	0.06 ± 0.01	0.08 ± 0.02	0.10 ± 0.02	0.10 ± 0.04	0.13 ± 0.02
MGDG (34:4)	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.01	0.02 ± 0.00
MGDG (34:3)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.00	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01
MGDG (34:2)	0.01 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.06 ± 0.02	0.00 ± 0.00
MGDG (34:1)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.00 ± 0.00
MGDG (36:6)	2.20 ± 0.78	2.44 ± 0.54	2.57 ± 0.32	3.93 ± 0.40	2.12 ± 0.48	3.84 ± 0.39
MGDG (36:5)	1.05 ± 0.37	0.56 ± 0.12	1.23 ± 0.16	0.49 ± 0.04	1.69 ± 0.40	0.54 ± 0.06
MGDG (36:4)	0.24 ± 0.07	0.21 ± 0.04	0.27 ± 0.04	0.44 ± 0.05	0.43 ± 0.09	0.39 ± 0.04
MGDG (36:3)	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.04 ± 0.01

tidylinositol (PI) species were mainly represented by 34:3 and 34:2 acyl species (Table 4). Phosphatidylglycerol (PG) and phosphatidylserine (PS) were in minority (data not shown).

The detailed analysis showed that decrease in PC species containing two unsaturated acyl species (36:5 and 36:6) and increase in 36:2 species were induced by MeJA treatment (Table 4). The minor phospholipids PG and PS were not substantially modified (data not shown). A drop in the ratio of compounds with 18:3 to total PI and PE was also observed during MeJA treatment (Table 4).

The lysophospholipids, LPG (16:0, 18:3, 18:2, 18:1) and the most abundant LPC (16:0, 18:3, 18:2, 18:1) and LPE (16:0, 18:3, 18:2, 18:1) were quantified in cultures, but MeJA did not induce changes in these lipid classes (Table 5).

High contents of phosphatidic acid (PA) were detected in cultures, the predominant molecular species being 34:3, 34:2, 36:5 and 36:4, which indicated that PA species were generated from either PC or PE. An increase in the unsaturated 36:3 and 36:2 species were observed at later time during elicitation (Table 5).

The presence of LPs and the high content of PA indicates a high basal level of phospholipase D (PLD) activity in cultures and, hence, a high phospholipid turnover. In fact, a strong basal PLD activity was detected in suspensions previously and a two-fold increase of PLD activity was produced shortly after MeJA treatment (Madrid and Corchete 2010). In the same report, it was also demonstrated that continuous generation of PA was necessary for silymarin release into the culture medium. In the present work, a high PA production was detected through the studied period. However, no significant increase in PA content was detected in the presence of MeJA. Yang *et al*. (2008) also observed that elicitation of *Taxus cuspidata* with MeJA did not markedly alter LPC and PA production and the authors suggested that the phospholipase A/D pathway might not contribute to the enhancement of taxol production commonly observed in MeJA-induced taxus cells.

Maybe alterations of specific molecular species during elicitation are more important than total changes.

Table 4. Individual molecular species of major glycerophospholipids [nmol mg^{-1}(d.m.)] in *Silybum marianum* cells treated with 100 μM MeJA for 8, 24 and 48 h. Means ± SD, $n = 5$.

Compound	Control 8	MeJA 8	Control 24	MeJA 24	Control 48	MeJA 48
PC (34:3)	4.81 ± 1.37	4.42 ± 0.75	5.10 ± 0.59	3.27 ± 0.28	4.27 ± 0.81	3.12 ± 0.43
PC (34:2)	4.30 ± 1.21	3.59 ± 0.63	4.38 ± 0.55	3.09 ± 0.30	4.27 ± 0.84	3.19 ± 0.43
PC (34:1)	2.69 ± 0.77	2.43 ± 0.43	2.76 ± 0.35	3.82 ± 0.35	1.78 ± 0.38	3.26 ± 0.51
PC (36:6)	2.97 ± 0.78	2.37 ± 0.39	3.29 ± 0.53	1.42 ± 0.13	2.28 ± 0.52	1.23 ± 0.20
PC (36:5)	5.36 ± 1.26	4.28 ± 0.67	5.93 ± 0.92	3.35 ± 0.39	5.22 ± 1.26	3.60 ± 0.62
PC (36:4)	5.32 ± 1.20	4.38 ± 0.67	6.02 ± 0.95	4.80 ± 0.56	4.88 ± 1.17	4.95 ± 0.89
PC (36:3)	3.18 ± 0.76	2.76 ± 0.44	3.64 ± 0.58	3.90 ± 0.44	2.69 ± 0.70	4.01 ± 0.91
PC (36:2)	1.30 ± 0.33	1.19 ± 0.19	1.44 ± 0.24	2.81 ± 0.35	0.88 ± 0.23	2.67 ± 0.54
PC (38:4)	0.20 ± 0.08	0.09 ± 0.01	0.13 ± 0.02	0.07 ± 0.00	0.08 ± 0.02	0.05 ± 0.01
PC (38:3)	0.18 ± 0.08	0.10 ± 0.01	0.13 ± 0.02	0.10 ± 0.01	0.09 ± 0.02	0.08 ± 0.01
PC (38:2)	0.14 ± 0.07	0.09 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.06 ± 0.02	0.09 ± 0.02
PE (34:3)	1.48 ± 0.48	2.45 ± 0.43	1.12 ± 0.49	1.95 ± 0.36	1.30 ± 0.36	1.79 ± 0.30
PE (34:2)	2.52 ± 0.79	3.71 ± 0.68	3.10 ± 0.81	3.48 ± 0.76	3.01 ± 0.83	3.71 ± 0.57
PE (34:1)	0.37 ± 0.11	0.55 ± 0.10	0.71 ± 0.10	1.14 ± 0.25	0.29 ± 0.07	1.19 ± 0.20
PE (36:6)	0.70 ± 0.23	0.93 ± 0.16	0.85 ± 0.18	0.50 ± 0.09	0.56 ± 0.13	0.46 ± 0.07
PE (36:5)	1.54 ± 0.50	2.15 ± 0.38	2.14 ± 0.39	1.62 ± 0.26	1.84 ± 0.41	1.72 ± 0.23
PE (36:4)	1.41 ± 0.46	2.15 ± 0.39	2.89 ± 0.37	2.19 ± 0.38	2.04 ± 0.50	2.68 ± 0.34
PE (36:3)	0.42 ± 0.14	0.63 ± 0.11	0.60 ± 0.09	0.90 ± 0.16	0.29 ± 0.11	1.07 ± 0.15
PE (36:2)	0.09 ± 0.03	0.14 ± 0.02	0.15 ± 0.02	0.28 ± 0.05	0.02 ± 0.02	0.30 ± 0.03
PE (40:2)	0.05 ± 0.02	0.10 ± 0.02	0.07 ± 0.01	0.06 ± 0.02	0.01 ± 0.01	0.08 ± 0.01
PE (42:3)	0.21 ± 0.07	0.24 ± 0.03	0.27 ± 0.03	0.17 ± 0.05	0.08 ± 0.03	0.15 ± 0.02
PE (42:2)	0.46 ± 0.13	0.53 ± 0.08	0.56 ± 0.08	0.41 ± 0.20	0.11 ± 0.07	0.52 ± 0.06
PI (34:3)	2.10 ± 0.68	1.52 ± 0.30	2.81 ± 0.34	1.04 ± 0.10	2.14 ± 0.48	0.65 ± 0.08
PI (34:2)	6.39 ± 1.98	6.96 ± 1.42	7.83 ± 0.96	8.73 ± 0.85	6.40 ± 1.49	8.27 ± 1.08
PI (34:1)	0.15 ± 0.04	0.22 ± 0.04	0.25 ± 0.03	0.36 ± 0.03	0.17 ± 0.04	0.22 ± 0.03
PI (36:6)	0.20 ± 0.06	0.17 ± 0.03	0.24 ± 0.03	0.10 ± 0.01	0.14 ± 0.03	0.08 ± 0.01
PI (36:5)	0.40 ± 0.12	0.41 ± 0.08	0.50 ± 0.05	0.35 ± 0.04	0.33 ± 0.07	0.35 ± 0.04
PI (36:4)	0.43 ± 0.13	0.44 ± 0.09	0.55 ± 0.05	0.40 ± 0.04	0.35 ± 0.07	0.42 ± 0.04
PI (36:3)	0.28 ± 0.09	0.29 ± 0.05	0.35 ± 0.04	0.34 ± 0.03	0.21 ± 0.05	0.31 ± 0.04
PI (36:2)	0.21 ± 0.06	0.25 ± 0.05	0.24 ± 0.03	0.56 ± 0.05	0.19 ± 0.04	0.45 ± 0.06

The detailed analysis showed that percentages of 36:6 PC and 36:5 PC and PE species were lower in the MeJA-treated cells than in the control, suggesting that the decrease of these species might resulted from a preference or partial accessibility of unsaturated species to hydrolytic enzymes, like PLD.

The understanding of intercellular and intracellular plant secondary metabolite trafficking processes in plants is receiving considerable attention. Many studies imply that membrane transporters, particularly ABC transporters, have significant roles in sequestering metabolites within particular intracellular compartments and/or exporting them to the apoplastic space (Yazaki *et al*. 2008). Recent studies have also revealed the existence of vesicle associated and SNARE protein-mediated exocytosis pathways engaged in the secretion of secondary metabolites (Kwon *et al*. 2011). Substantial efforts are being made to demonstrate the importance of lipids and lipid-modifying enzymes in various membrane trafficking processes. Among bioactive lipids, phosphatidic acid (PA) is an attractive candidate due to its ability to promote membrane fusion by changing membrane topology. Although the role of lipids in exocytosis is intuitive to date and the actual function of PA in secretion remains unknown, it has been demonstrated that PA facilitates a late event in the granule fusion pathway during exocytosis by altering membrane curvature and promoting hemi-fusion. (Zeniu-Meyer *et al*. 2007). In this context Wang *et al*. (2006) hypothesized that in plant-stress interaction the timing and location of PA production and appropriate cellular concentrations are important determinants of PA function.

Exploring the mechanisms for silymarin export across plasma membrane and subsequently characterizing the potential biochemical factors involved may lead to rational solutions for improving the biotechnological production of this secondary metabolite.

Table 5. Individual molecular species of LP and PA [nmol mg^{-1}(d.m.)] in *Silybum marianum* cells treated with 100 μM MeJA for 8, 24 and 48 h. Means ± SD, $n = 5$.

Compound	Control 8	MeJA 8	Control 24	MeJA 24	Control 48	MeJA 48
LPG(16:1)	0.000 ± 0.000	0.003 ± 0.004	0.002 ± 0.002	0.008 ± 0.008	0.007 ± 0.009	0.016 ± 0.008
LPG(16:0)	0.025 ± 0.012	0.024 ± 0.016	0.033 ± 0.015	0.029 ± 0.011	0.031 ± 0.024	0.016 ± 0.009
LPG(18:3)	0.005 ± 0.003	0.003 ± 0.002	0.009 ± 0.008	0.011 ± 0.004	0.009 ± 0.006	0.008 ± 0.005
LPG(18:2)	0.006 ± 0.003	0.006 ± 0.004	0.011 ± 0.005	0.010 ± 0.007	0.004 ± 0.003	0.013 ± 0.008
LPG(18:1)	0.002 ± 0.001	0.001 ± 0.001	0.000 ± 0.000	0.001 ± 0.001	0.000 ± 0.000	0.001 ± 0.001
Total LPG	0.038 ± 0.015	0.036 ± 0.021	0.054 ± 0.018	0.059 ± 0.013	0.051 ± 0.039	0.054 ± 0.018
LPC(16:1)	0.002 ± 0.002	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.001	0.000 ± 0.000	0.000 ± 0.000
LPC(16:0)	0.057 ± 0.024	0.036 ± 0.014	0.050 ± 0.014	0.044 ± 0.002	0.037 ± 0.013	0.027 ± 0.006
LPC(18:3)	0.127 ± 0.052	0.087 ± 0.028	0.106 ± 0.025	0.069 ± 0.004	0.057 ± 0.023	0.037 ± 0.007
LPC(18:2)	0.118 ± 0.050	0.075 ± 0.024	0.094 ± 0.020	0.071 ± 0.007	0.071 ± 0.027	0.047 ± 0.007
LPC(18:1)	0.079 ± 0.033	0.052 ± 0.018	0.061 ± 0.014	0.088 ± 0.008	0.031 ± 0.014	0.053 ± 0.011
LPC(18:0)	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.003 ± 0.001	0.002 ± 0.001	0.003 ± 0.001
Total LPC	0.384 ± 0.160	0.250 ± 0.084	0.313 ± 0.072	0.275 ± 0.020	0.198 ± 0.078	0.167 ± 0.032
LPE(16:1)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
LPE(16:0)	0.056 ± 0.019	0.050 ± 0.011	0.070 ± 0.013	0.044 ± 0.025	0.013 ± 0.012	0.047 ± 0.013
LPE(18:3)	0.040 ± 0.013	0.044 ± 0.007	0.055 ± 0.015	0.039 ± 0.004	0.026 ± 0.006	0.021 ± 0.006
LPE(18:2)	0.078 ± 0.027	0.079 ± 0.019	0.108 ± 0.028	0.089 ± 0.014	0.067 ± 0.021	0.057 ± 0.011
LPE(18:1)	0.015 ± 0.006	0.018 ± 0.004	0.019 ± 0.007	0.032 ± 0.005	0.009 ± 0.003	0.017 ± 0.005
Total LPE	0.189 ± 0.062	0.192 ± 0.040	0.251 ± 0.058	0.204 ± 0.037	0.116 ± 0.040	0.142 ± 0.032
PA(32:0)	0.005 ± 0.004	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.002	0.003 ± 0.001	0.005 ± 0.000
PA(34:6)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
PA(34:5)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
PA(34:4)	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.001
PA(34:3)	0.203 ± 0.119	0.144 ± 0.037	0.185 ± 0.031	0.149 ± 0.022	0.144 ± 0.055	0.177 ± 0.018
PA(34:2)	0.314 ± 0.193	0.203 ± 0.052	0.265 ± 0.032	0.242 ± 0.033	0.213 ± 0.070	0.300 ± 0.024
PA(34:1)	0.076 ± 0.037	0.063 ± 0.016	0.074 ± 0.012	0.103 ± 0.016	0.052 ± 0.021	0.154 ± 0.015
PA(36:6)	0.069 ± 0.036	0.057 ± 0.017	0.077 ± 0.012	0.048 ± 0.009	0.049 ± 0.021	0.050 ± 0.005
PA(36:5)	0.164 ± 0.084	0.132 ± 0.038	0.176 ± 0.022	0.126 ± 0.024	0.146 ± 0.058	0.161 ± 0.016
PA(36:4)	0.159 ± 0.072	0.139 ± 0.039	0.183 ± 0.031	0.161 ± 0.024	0.150 ± 0.051	0.223 ± 0.024
PA(36:3)	0.086 ± 0.041	0.078 ± 0.021	0.096 ± 0.012	0.109 ± 0.020	0.073 ± 0.026	0.168 ± 0.015
PA(36:2)	0.031 ± 0.015	0.030 ± 0.009	0.033 ± 0.006	0.059 ± 0.013	0.020 ± 0.009	0.100 ± 0.011
Total PA	1.109 ± 0.597	0.850 ± 0.229	1.095 ± 0.153	1.002 ± 0.154	0.850 ± 0.310	1.339 ± 0.116

References

Capataz-Tafur, J., Hernández-Sánchez, A., Rodriguez-Monroy, M., Trejo-Tapia, G., Sepúlveda Jiménez, G.: Sucrose induces arabinogalactan protein secretion by *Beta vulgaris* L. cell suspension cultures. - Acta Physiol. Plant. **32**: 757-764, 2010.

Corrado, G., Agrelli, D., Roccco, M., Basile, B., Marra, M., Rao, R.: Systemin-inducible defence against pests is costly in tomato. - Biol. Plant. **55**: 305-311, 2011.

Flora, K., Hahn, M., Benner, K.: Milk thistle (*Silybum marianum*) for the therapy of liver disease. - Amer. J. Gastroenterology **93**: 139-143, 1998.

Gutiérrez-Carbajal, M.G., Monforte-González, M., Miranda-Ham, M de L., Godoy-Hernández, G., Vázquez-Flota, F.: Induction of capsaicinoid synthesis in *Capsicum chinense* cell cultures by salicylic acid or methyl jasmonate. - Biol. Plant. **54**: 430-434, 2010.

Kim, H.J., Chen, F., Wang, X., Rajpakse, N.C.: Effect of methyl jasmonate on secondary metabolites of sweet basil (*Occimum basilicum* L.). - J. Agr. Food Chem. **54**: 2327-2332, 2006.

Kwon, C., Bednarek, P., Schulze-Lefert, P.: Secretory pathways in plant immune responses. - Plant Physiol. **147**: 1575-1583, 2008.

Larkindale, J., Huang, B.: Changes of lipid composition and saturation level in leaves and roots for heat-stressed and heat acclimated creeping bentgrass (*Agrostis stolonifera*). - Environ. exp. Bot. **51**: 57-67, 2004.

Luo, Y., Li, F., Wang, G.P., Yang, X.H., Wang, W.: Exogenously-supplied trehalose protects thylakoid membranes of winter wheat from heat-induced damage. - Biol. Plant. **55**: 495-501, 2010.

Madrid, E., Corchete, P.: Silymarin secretion and its elicitation by methyl jasmonate in cell cultures of *Silybum marianum* is mediated by phospholipase D-phosphatidic acid. - J. exp.

Bot. **61**: 747-754, 2010.
Meijer, H.J.G., Munnik, T.: Phospholipid-based signaling in plants. - Annu. Rev. Plant Biol. **54**: 265-306, 2003.
Munnik, T.: Phosphatidic acid: an emerging plant lipid second messenger. - Trends Plant Sci. **6**: 227-233, 2001.
Profotova, B., Burketova, L., Novotna, Z., Martinec, J., Valentova, O.: Involvement of phospholipases C and D in early responses to SAR and ISR inducers in *Brassica napus* plants. - Plant Physiol. Biochem. **44**: 143-151, 2005.
Rhadika, V., Kost, C., Boland, W., Heil, M.: The role of jasmonates in floral nectar secretion. - PlosONE **5**: e9265, 2010.
Repka, V., Fischerová, I., Šilhárová, K.: Methyl jasmonate is a potent elicitor of multiple defense responses in grapevine leaves and cell suspension cultures.- Biol. Plant. **48**: 273-283, 2004.
Ruiz-May, E., Galaz-Avalos, R.M., Loyola-Vargas, V.M.: Differential secretion and accumulation of terpene indole alkaloids in hairy roots of *Catharanthus roseus* treated with methyl jasmonate. - Mol. Biotechnol. **41**: 278-285, 2009.
Salzman, R.A., Brady, J.A., Finlayson, S.A., Buchanan, C.D., Summer, E.J., Sun, F., Klein, P.E., Klein, R.R., Pratt, L.H., Cordonnier-Pratt, M.M., John, E., Mullet, J.E.: Transcriptional profiling of sorghum induced by methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid reveals cooperative regulation and novel gene responses. - Plant Physiol. **138**: 352-368, 2005.
Sánchez-Sampedro, M.A., Fernández-Tarrago, J., Corchete, P.: Yeast extract and methyl jasmonate-induced silymarin production in cell cultures of *Silybum marianum* (L.) Gaertn. - J. Biotechnol. **119**: 60-69, 2005a.
Sánchez-Sampedro, M.A., Fernández-Tarrago, J., Corchete, P.: Enhanced silymarin accumulation is related to calcium deprivation in cell suspension cultures of *Silybum marianum* (L.) Gaertn. - J. Plant Physiol. **162**: 1177-1182, 2005b.
Testerink, C., Munnik, T.: Phosphatidic acid: a multifunctional stress signaling lipid in plants. - Trends Plant Sci. **10**: 368-375, 2005.
Wang, X.M., Devaiah, S.P., Zhang, W.H., Welti, R.: Signalling functions of phosphatidic acid. - Progr. Lipid Res. **45**: 250-278, 2006.
Welti, R., Li, W., Li, M., Sang, Y., Biesiadaj, H., Zhou, H.E., Rajashekar, C.B., Williams, T.D., Wang, X.: Profiling membrane lipids in plant stress responses. - J. biol. Chem. **30**: 31994-32002, 2002.
Yamaguchi, T., Minami, E., Shibuya, N.: Activation of phospholipases by N-acetylchitooligosaccharide elicitor in suspension-cultured rice cells mediates reactive oxygen generation. - Physiol. Plant. **118**: 361-370, 2003.
Yang, S., Lu, S.H., Yuan, Y.J.: Lipidomic analysis reveals differential defense responses of *Taxus cuspidata* cells to two elicitors, methyl jasmonate and cerium (Ce^{4+}). - Biochim. biophys. Acta **1781**: 123-134, 2008.
Yang, S., Qiao, S.H., Lu, S.H., Yuan, Y.J.: Comparative lipidomics analysis of cellular development and apoptosis in two *Taxus* cell lines. - Biochim. biophys. Acta **1771**: 600-612, 2007.
Yazaki, K., Sugiyama, A., Morita, M., Shitan, N.: Secondary transport as an efficient membrane transport mechanism for plant secondary metabolites. - Phytochem. Rev. **7**: 513-524, 2008.
Zeniou-Meyer, M., Zabari, N., Ashery, U., Chasserot-Golaz, S., Haeberlé, A-M., Bailly, Y., Nakanishi, H., Neiman, A.-M., Du, G., Frohman, M.A., Bader, M.F., Vitale. N.: PLD1 production of phosphatidic acid at the plasma membrane promotes exocytosis of large dense-core granule at a late stage. - J. biol. Chem. **282**: 21746-21757, 2007.