

# The interplay between membrane lipids and phospholipase A family members in grapevine resistance against *Plasmopara viticola*

Gonçalo Laureano <sup>a</sup>, Joana Figueiredo <sup>abc\*</sup>, Ana Rita Cavaco <sup>a\*</sup>, Bernardo Duarte <sup>d</sup>, Isabel Caçador <sup>d</sup>, Rui Malhó <sup>a</sup>, Marta Sousa Silva <sup>bc</sup>, Ana Rita Matos <sup>a†</sup>, Andreia Figueiredo <sup>a†#</sup>

<sup>a</sup> *Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

<sup>b</sup> *Laboratório de FTICR e Espectrometria de Massa Estrutural, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

<sup>c</sup> *Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

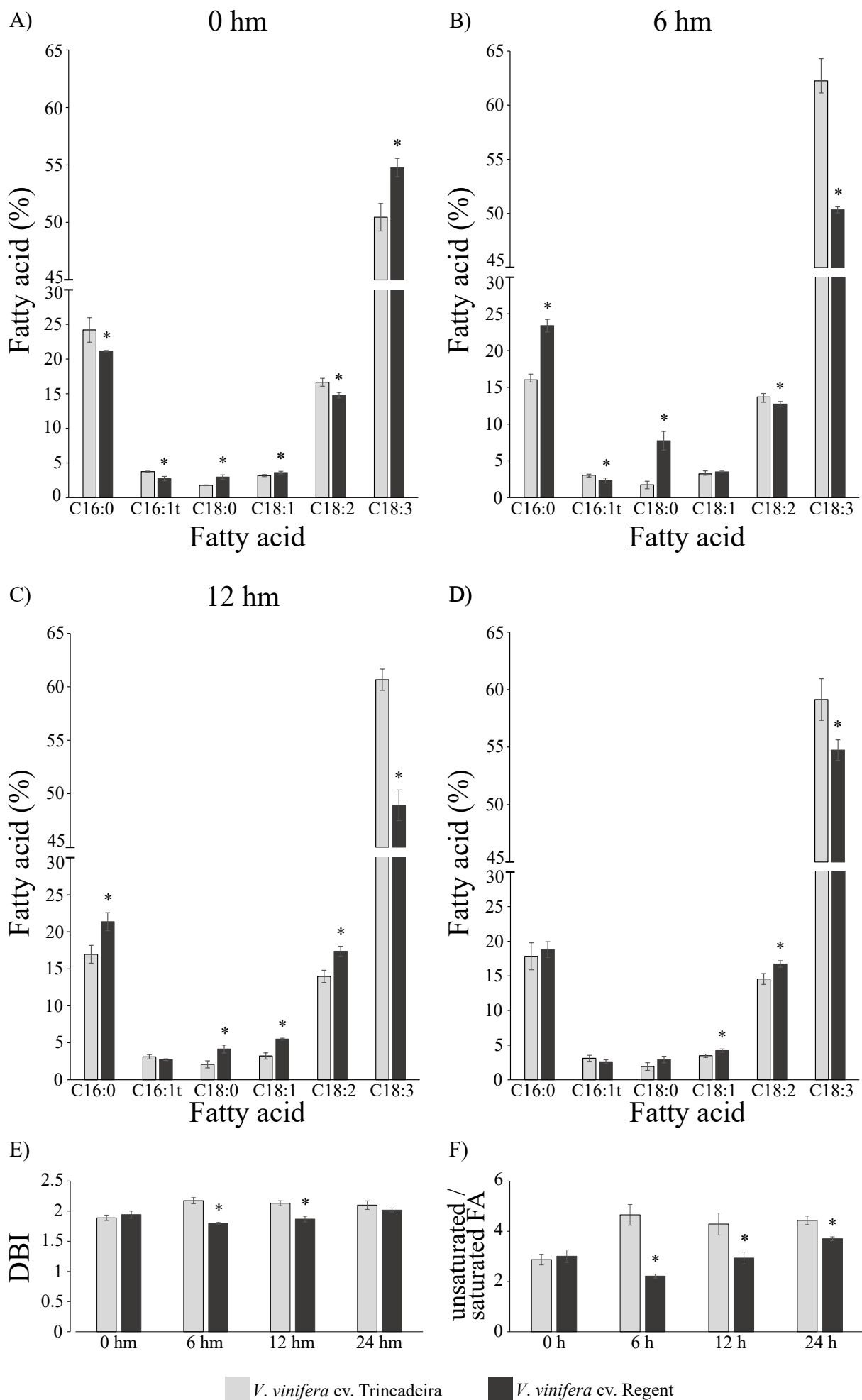
<sup>d</sup> *MARE - Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal*

\* These authors contributed equally in this paper

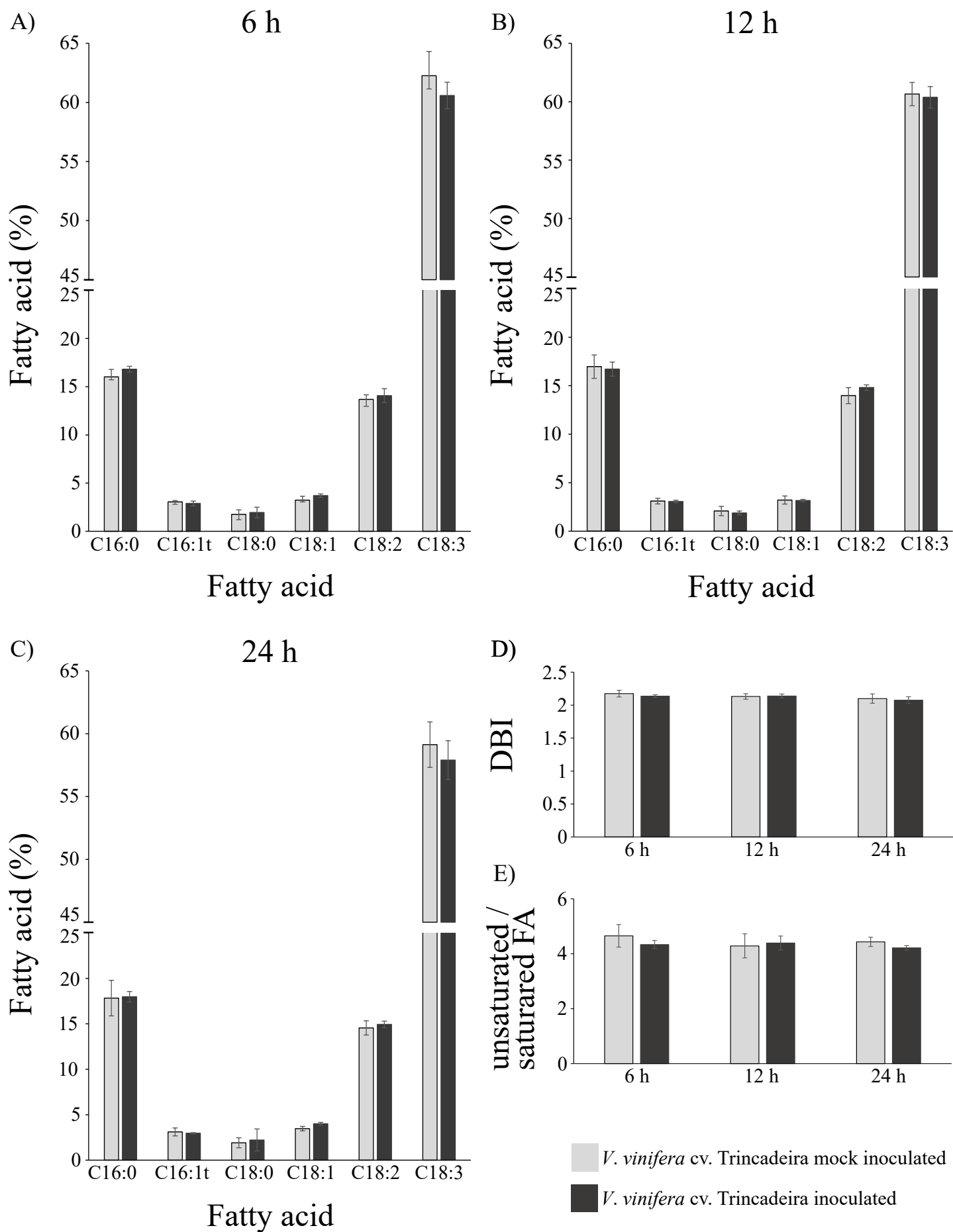
† These authors are co-senior authors in this paper

Corresponding author:

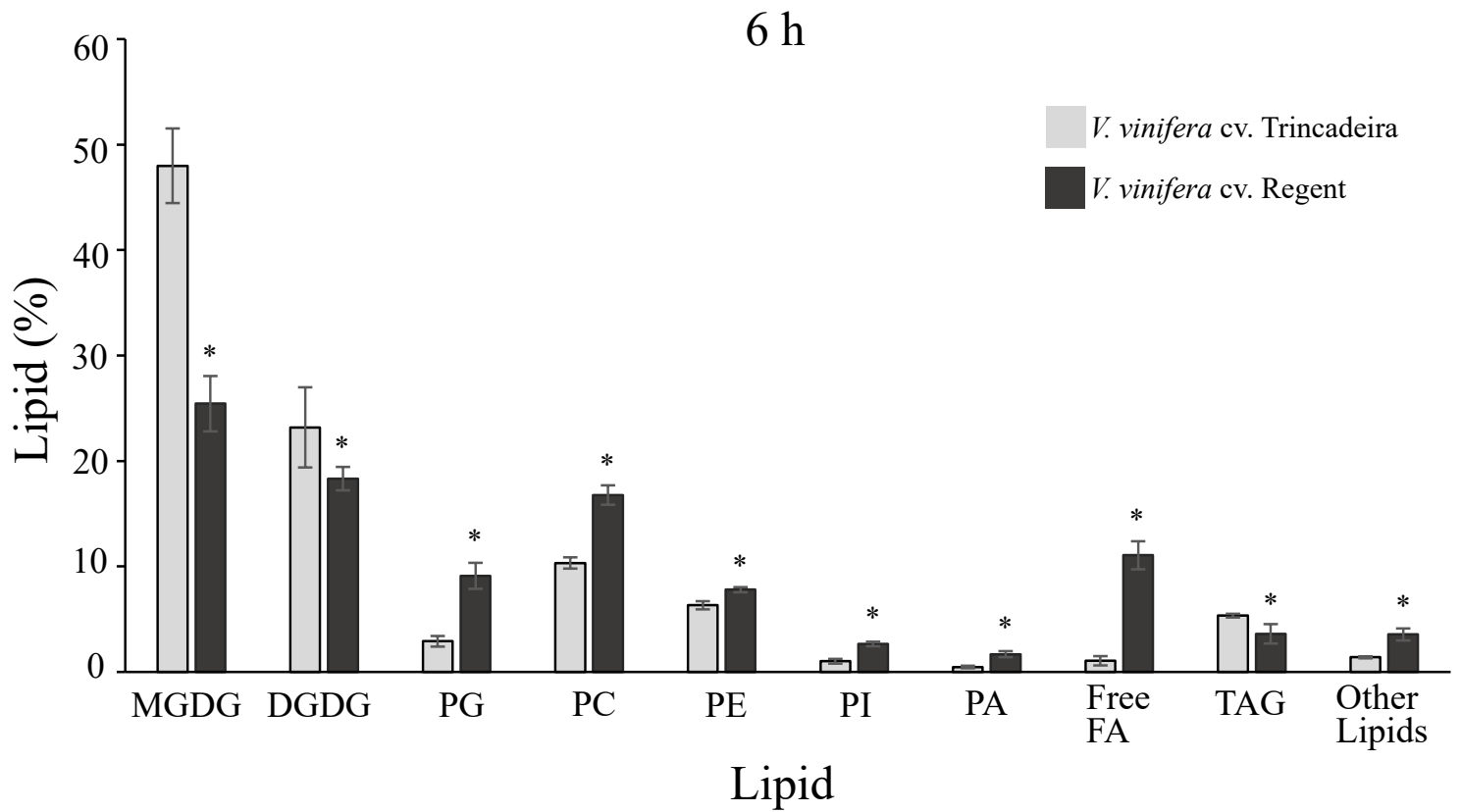
#Andreia Figueiredo, at Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, 1749-016 Lisboa, Portugal. e-mail address: [aafigueiredo@fc.ul.pt](mailto:aafigueiredo@fc.ul.pt)



**Supplementary Figure S1-** Fatty acid composition between *V. vinifera* cv. Trincadeira (light grey) and *V. vinifera* cv. Regent (dark grey) mock inoculated leaves at 0 (A), 6 (B), 12 (C) and 24 (D) hours; E) Double bound index (DBI); F) Ratio between unsaturated and saturated FA. Values correspond to average relative percentage  $\pm$  standard error, n = 4; Asterisks indicate significant differences ( $p < 0.05$ ).

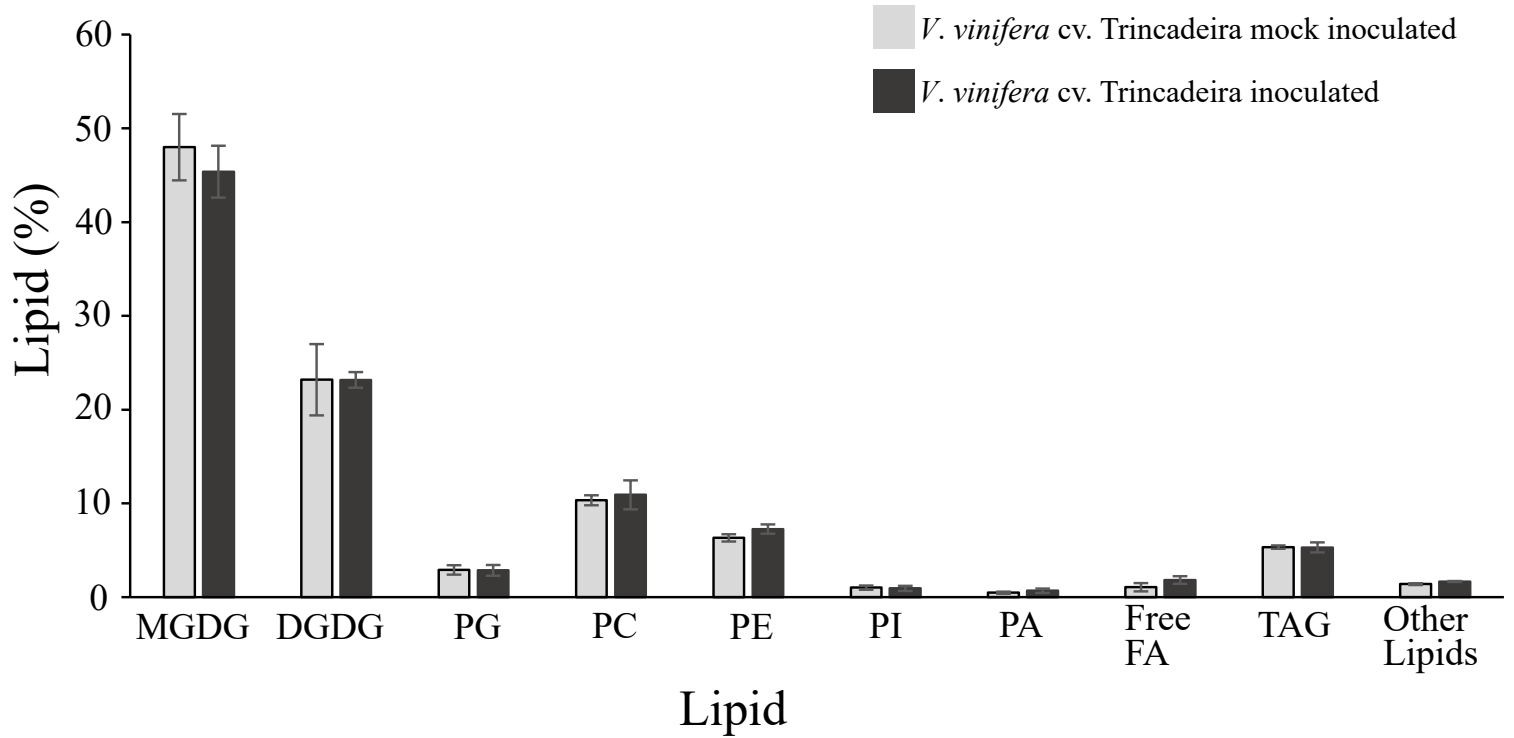


**Supplementary Figure S2** - Fatty acid composition of *V. vinifera* cv. Trincadeira mock inoculated (light grey) and inoculated (dark grey) leaves with *P. viticola* at 6 (A), 12 (B) and 24 (C) hours; D) Double bound index (DBI); E) Ratio between unsaturated and saturated FA. Values correspond to average relative percentage  $\pm$  standard error, n = 4; Asterisks indicate significant differences ( $p < 0.05$ ).

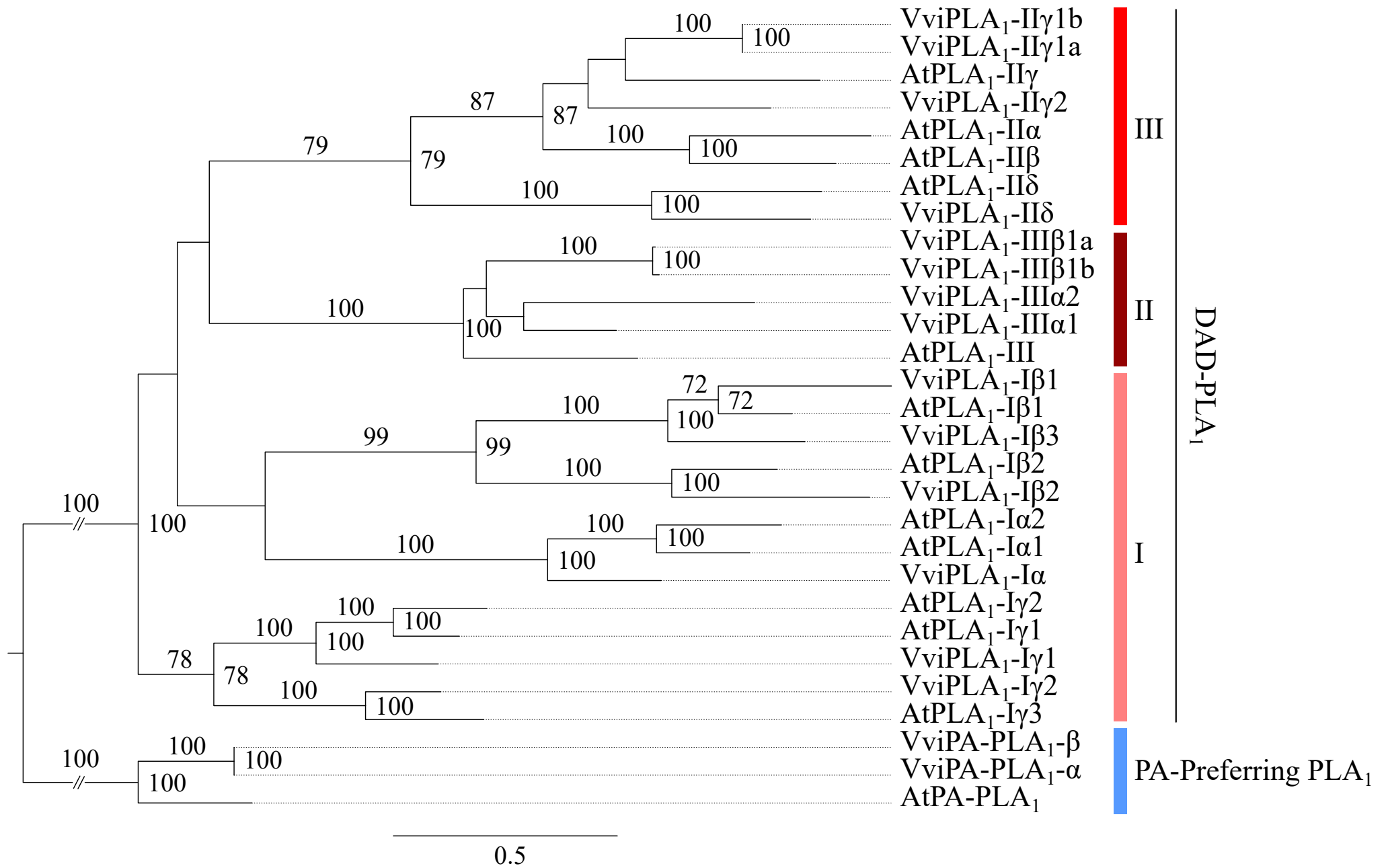


**Supplementary Figure S3** - Lipid composition of *V. vinifera* cv. Trincadeira (light grey) and Regent (dark grey) mock inoculated leaves at 6 hours. Values correspond to average relative percentage  $\pm$  standard error,  $n = 3$ ; Asterisks indicate significant differences ( $p < 0.05$ ). Abbreviations: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PI), phosphatidic acid (PA), free fatty acids (FA) and triacylglycerol (TAG).

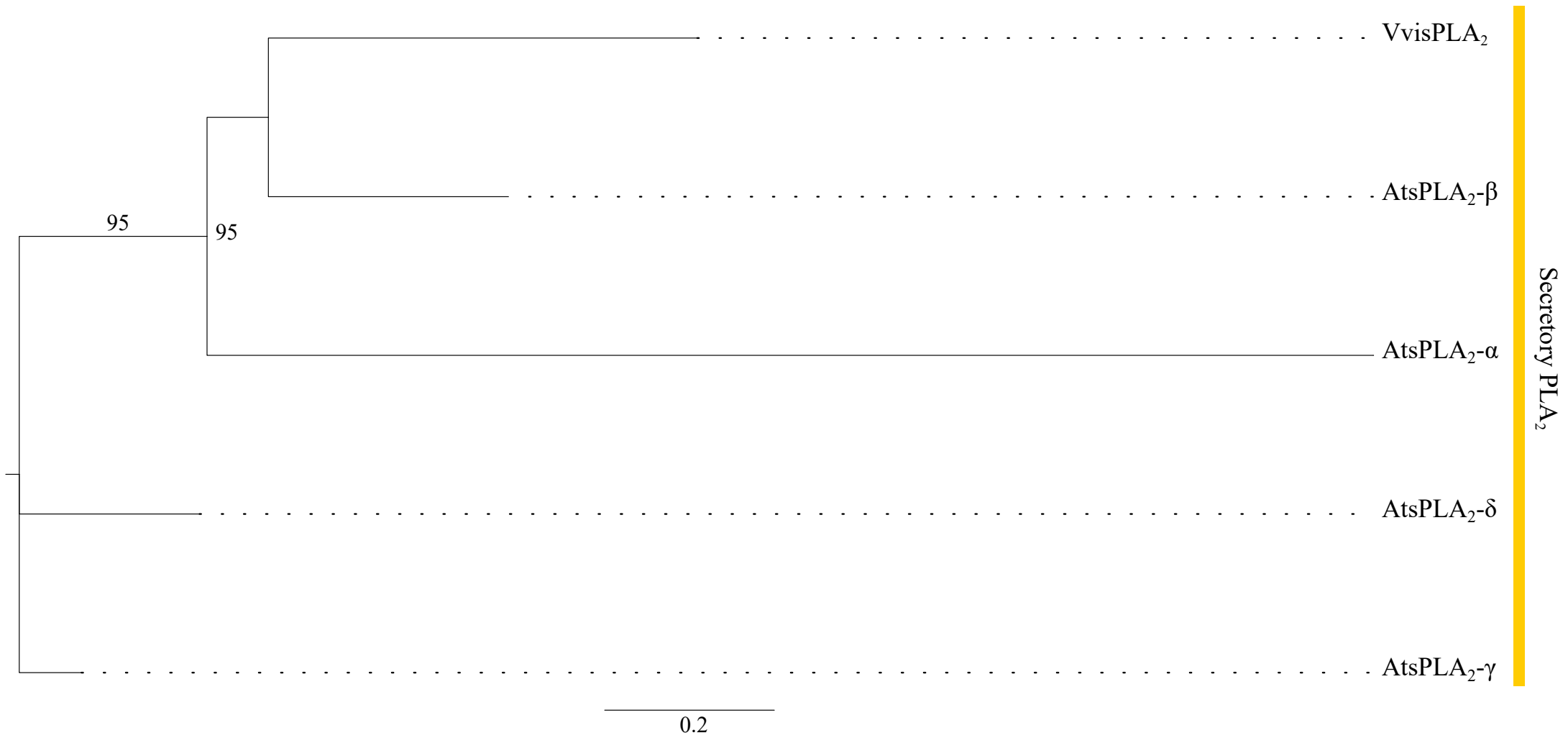
6 h



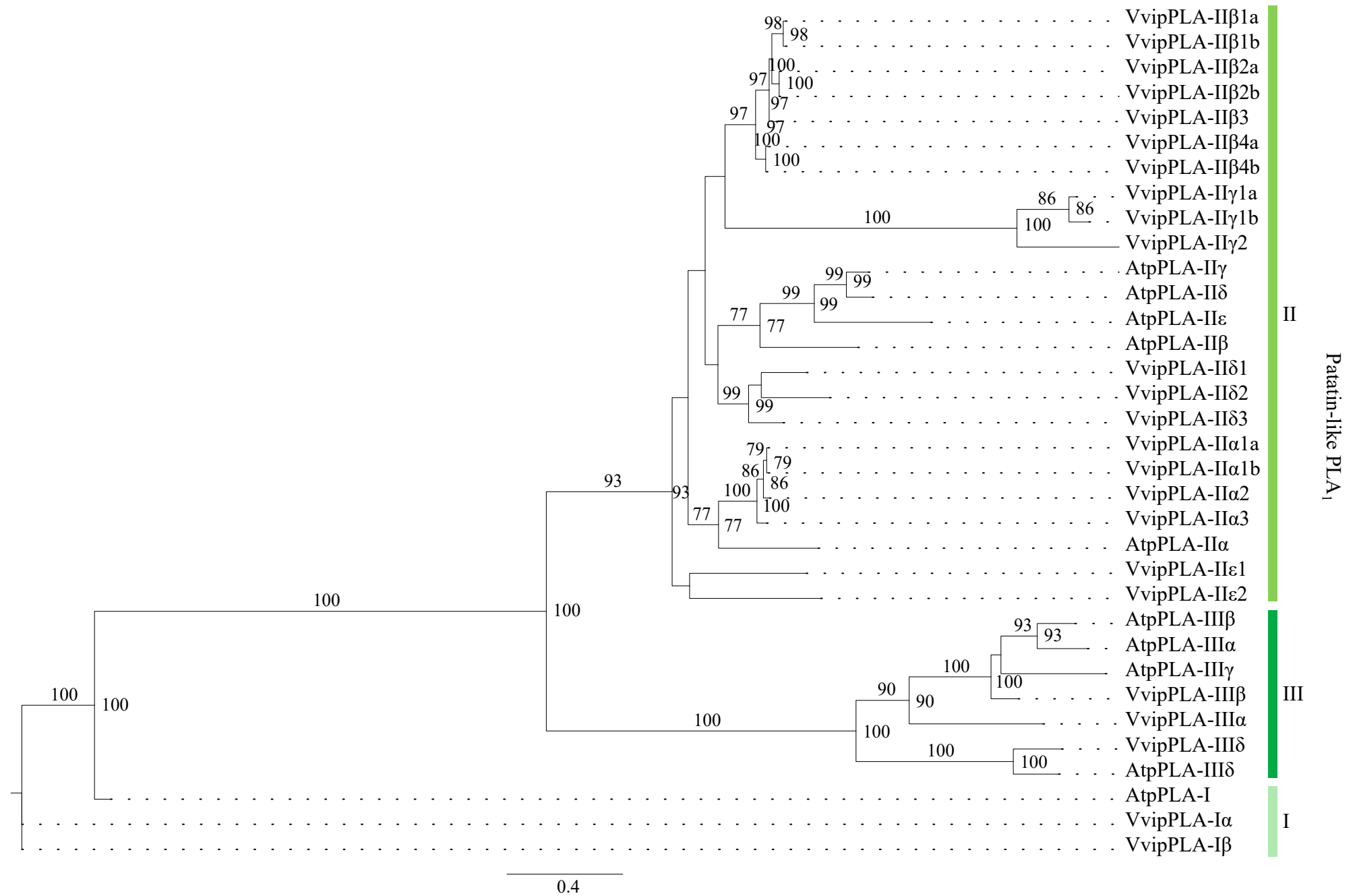
**Supplementary Figure S4** - Lipid composition of *V. vinifera* cv. Trincadeira mock inoculated (hm) and inoculated (hpi) leaves at 6 hours. Values correspond to average relative percentage  $\pm$  standard error,  $n = 3$ ; Asterisks indicate significant differences ( $p < 0.05$ ). Abbreviations: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PI), phosphatidic acid (PA), free fatty acids (FA) and triacylglycerol (TAG).



**Supplementary Figure S5** - Maximum likelihood phylogenetic tree of the 16 from *Vitis vinifera* PLA<sub>1</sub> proteins and 13 PLA<sub>1</sub> proteins from *Arabidopsis thaliana*. Scale bar represents the number of estimated changes per branch length. The root was truncated with double dash totalling 1.5 changes per branch length.

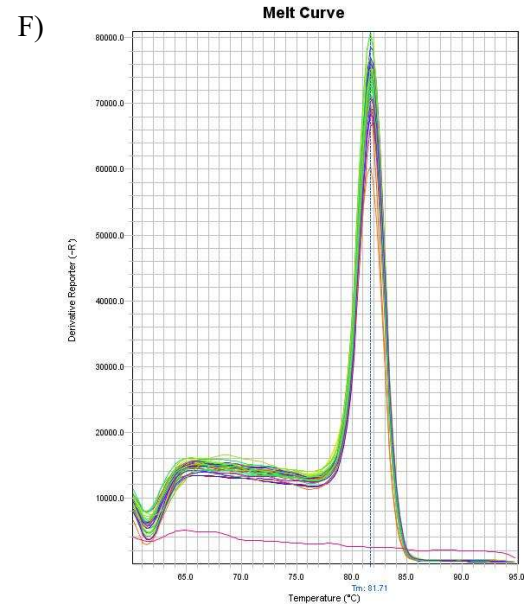
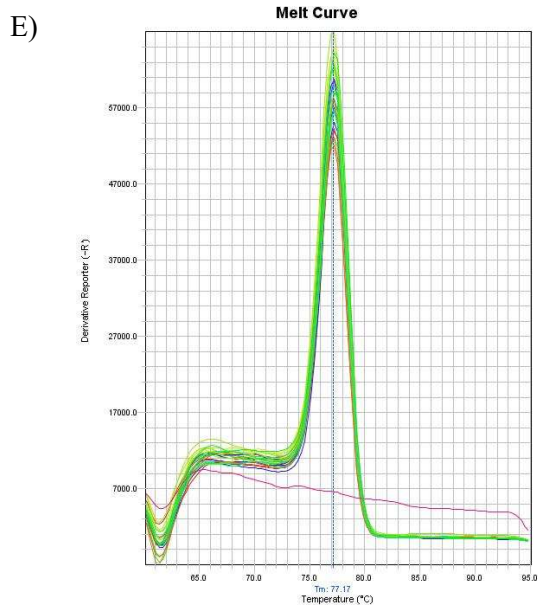
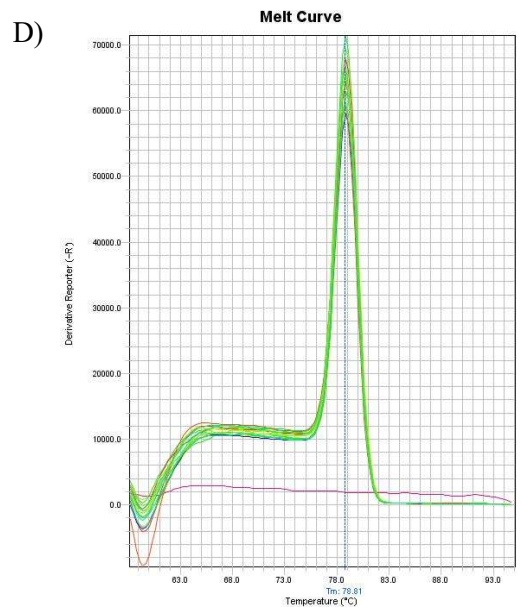
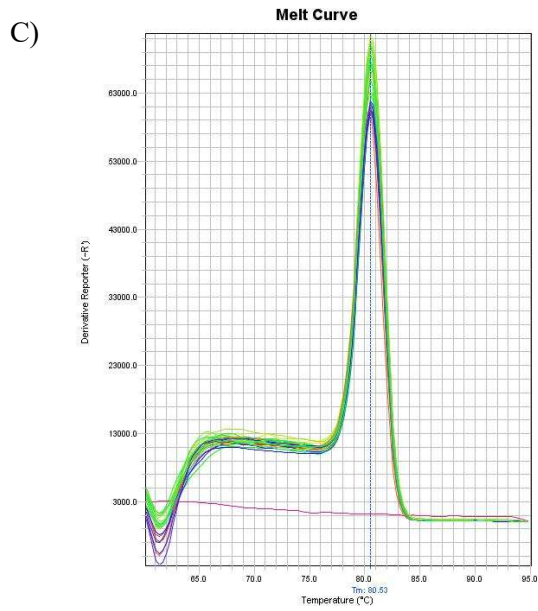
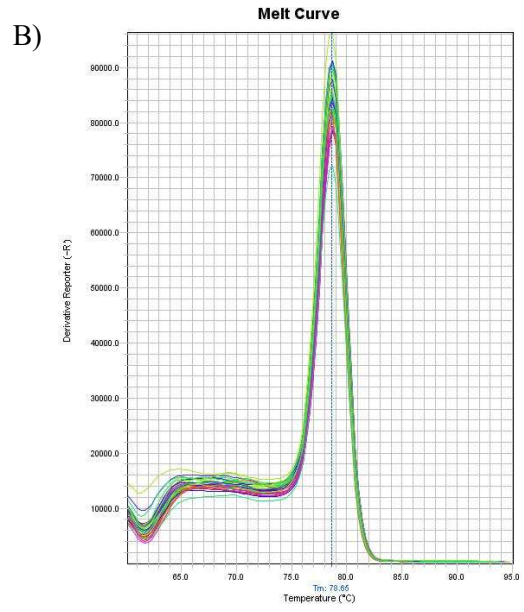
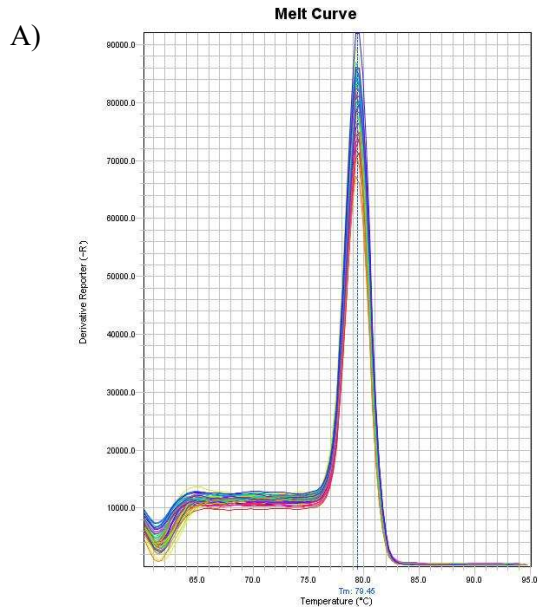


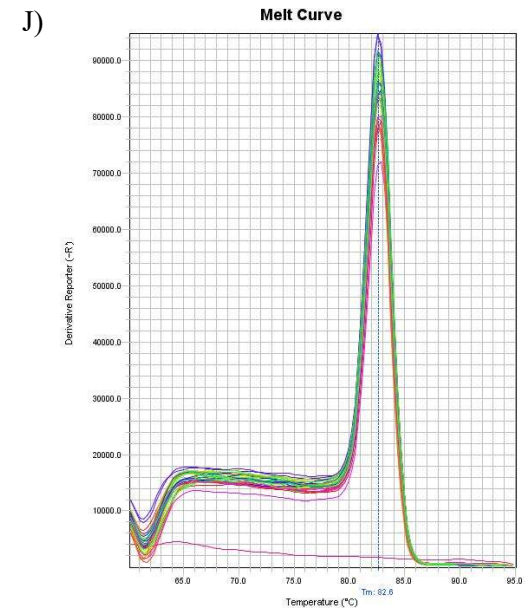
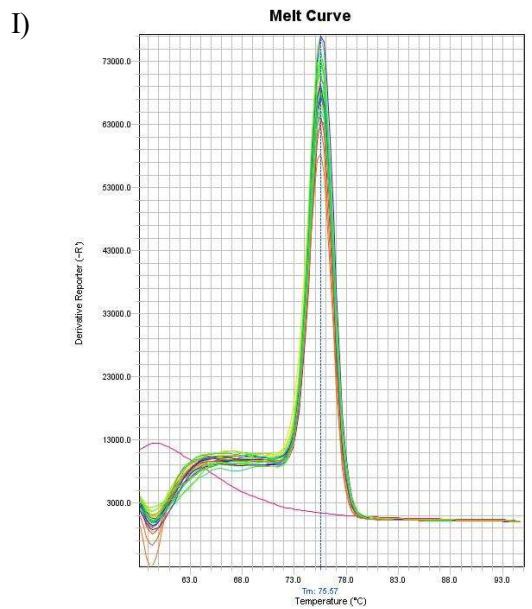
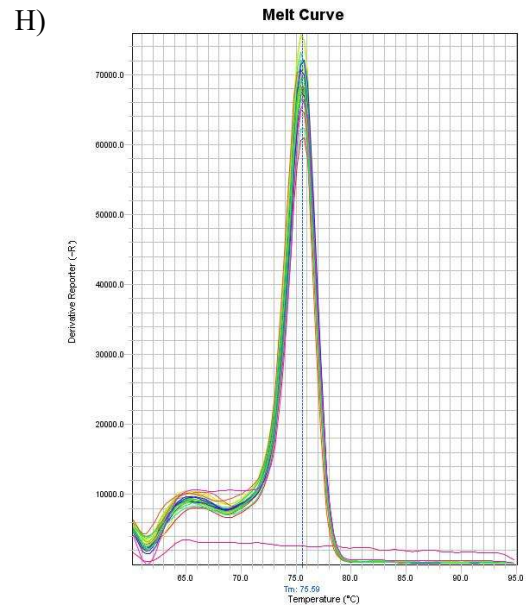
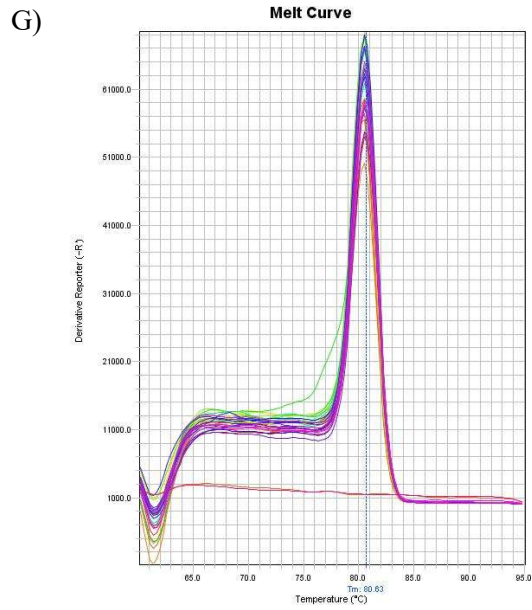
**Supplementary Figure S6** - Maximum likelihood phylogenetic tree of the 1 from *Vitis vinifera* secretory PLA proteins and 4 PLA proteins from *Arabidopsis thaliana*. Scale bar represents the number of estimated changes per branch length



**Supplementary Figure S7** - Maximum likelihood phylogenetic tree of the 24 from *Vitis vinifera* patatin-like PLA proteins and 10 PLA proteins from *Arabidopsis thaliana*. Scale bar represents the number of estimated changes per branch length.







**Supplementary Figure S8** - Melting curves of targeted genes. A) *EF1α*; B) *UBQ*; C) *VviPLA1-Iβ1*; D) *VviPLA1-Iγ1*; E) *VviPLA1-IIδ*; F) *VvisPLA2*; G) *VvipPLA-I*; H) *VvipPLA-IIβ*; I) *VvipPLA-IIδ2*; J) *VvipPLA-III*.

Trincadeira		C16:0	C16:1t	C18:0	C18:1	C18:2	C18:3	DBI	C18:3/18:2
MGDG	M	2.3	0	0.4	0.7	3.6	93.0	2.9	25.8
		±		±	±	±	±	±	±
	0.1		0.1	0.2	0.0	0.2	0.0	0.1	
	±		±	±	±	±	±	±	
DGDG	M	2.2	0	0.4	1.1	3.7	92.7	2.9	<b>25.0*</b>
		±		±	±	±	±	±	±
	0.4		0.0	0.2	0.1	0.5	0.0	<b>0.3</b>	
	±		±	±	±	±	±	±	
PC	M	20.4	0	2.1	1.1	3.0	73.5	2.3	25.4
		±		±	±	±	±	±	±
	4.2		0.7	0.2	0.4	5.4	0.2	4.9	
	±		±	±	±	±	±	±	
PE	M	20.5	0	1.6	1.0	2.5	74.3	2.3	29.6
		±		±	±	±	±	±	±
	1.0		0.1	0.1	0.1	0.8	0.0	0.7	
	±		±	±	±	±	±	±	
TAG	M	36.1	0	5.1	6.5	33.5	18.8	1.3	0.6
		±		±	±	±	±	±	±
	1.9		1.0	0.5	2.0	1.4	0.1	0.0	
	±		±	±	±	±	±	±	
PG	M	36.4	0	5.0	7.3	33.6	17.7	1.3	<b>0.5*</b>
		±		±	±	±	±	±	±
	4.7		0.8	0.5	3.2	1.8	0.1	<b>0.0</b>	
	±		±	±	±	±	±	±	
Other Lipids	M	42.9	0	3.7	1.2	40.7	12.6	1.2	0.3
		±		±	±	±	±	±	±
	6.2		1.3	1.6	3.8	2.2	0.2	0.0	
	±		±	±	±	±	±	±	
PA	M	40.2	0	2.9	2.2	41.8	12.9	1.2	0.3
		±		±	±	±	±	±	±
	3.3		0.0	0.2	2.6	0.9	0.1	0.0	
	±		±	±	±	±	±	±	
PI	M	14.4	0	4.8	4.4	42.4	34.0	1.9	0.8
		±		±	±	±	±	±	±
	0.1		0.3	0.2	0.1	0.3	0.0	0.0	
	±		±	±	±	±	±	±	
Free FA	M	13.7	0	4.3	<b>5.2*</b>	41.3	34.3	1.9	0.8
		±		±	±	±	±	±	±
	0.8		0.6	<b>0.6</b>	0.8	0.6	0.0	0.0	
	±		±	±	±	±	±	±	
MGDG	M	34.7	34.4	3.8	13.8	4.1	8.3	0.4	2.0
		±	±	±	±	±	±	±	±
	5.3	2.4	0.6	2.5	0.8	2.9	0.1	0.3	
	±	±	±	±	±	±	±	±	
DGDG	M	37.6	30.7	3.4	16.8	4.2	8.2	0.4	1.7
		±	±	±	±	±	±	±	±
	6.5	3.5	1.9	2.8	2.5	2.6	0.1	0.4	
	±	±	±	±	±	±	±	±	
PC	M	32.1	0	19.8	12.8	25.0	16.5	1.1	0.6
		±		±	±	±	±	±	±
	4.2		4.2	3.3	4.2	4.0	0.2	0.1	
	±		±	±	±	±	±	±	
PE	M	40.3	0	<b>30.3*</b>	10.0	14.5	7.7	0.6	0.7
		±		±	±	±	±	±	±
	5.0		<b>3.2</b>	1.9	4.0	6.3	0.2	0.0	
	±		±	±	±	±	±	±	
TAG	M	28.9	0	13.9	0	47.3	7.9	1.2	0.3
		±		±	±	±	±	±	±
	13.3		18.2		25.3	11.2	0.7	0.3	
	±		±	±	±	±	±	±	
PG	M	24.2	0	13.7	4.7	31.9	17.1	1.4	0.4
		±		±	±	±	±	±	±
	21.8		6.0	4.9	16.2	8.9	0.6	0.3	
	±		±	±	±	±	±	±	
Other Lipids	M	34.4	0	11.0	3.8	19.6	27.4	1.3	1.6
		±		±	±	±	±	±	±
	3.1		7.0	3.4	3.1	2.5	0.1	0.3	
	±		±	±	±	±	±	±	
PA	M	33.6	0	14.8	4.3	18.7	25.4	1.1	1.6
		±		±	±	±	±	±	±
	3.9		6.0	1.8	1.9	4.2	0.1	0.3	
	±		±	±	±	±	±	±	
Free FA	M	34.7	0	53.1	5.8	4.4	2.7	0.2	0.8
		±		±	±	±	±	±	±
	4.4		5.1	4.2	5.0	5.2	0.3	0.1	
	±		±	±	±	±	±	±	
MGDG	M	39.6	0	51.3	5.5	5.2	2.0	0.2	0.6
		±		±	±	±	±	±	±
	2.4		2.7	0.6	0.7	3.9	0.1	0.7	
	±		±	±	±	±	±	±	

**Supplementary Table S1** - Fatty acids content in each lipid class *V. vinifera* cv. Trincadeira mock inoculated (M) and inoculated (I) leaves with *P. viticola*. Values correspond to average relative percentage ± standard error, n = 3; Asterisks indicate significant differences (p<0.05).

Regent		C16:0	C16:1t	C18:0	C18:1	C18:2	C18:3	DBI	C18:3/18:2
MGDG	M	6.5		1.9	2.2	3.9	85.5	2.7	22.1
		±	0	±	±	±	±	±	±
	0.7		0.2	1.4	0.1	2.0	0.0	1.0	
	<b>3.2*</b>		<b>0.8*</b>	<b>0.6*</b>	<b>3.4*</b>	<b>92.0*</b>	<b>2.8*</b>	<b>26.9*</b>	
DGDG	I	±	0	±	±	±	±	±	±
		<b>0.1</b>		<b>0.0</b>	<b>0.1</b>	<b>0.1</b>	<b>0.2</b>	<b>0.0</b>	<b>0.6</b>
	24.2		3.4	1.4	3.0	67.9	2.1	23.7	
	±	0	±	±	±	±	±	±	
PC	M	1.1		0.4	0.1	0.2	1.2	0.1	2.4
		22.0		<b>1.8*</b>	<b>0.8*</b>	<b>1.9*</b>	<b>73.5*</b>	<b>2.2*</b>	<b>38.7*</b>
	±	0	±	±	±	±	±	±	
	<b>0.7</b>		<b>0.1</b>	<b>0.1</b>	<b>0.0</b>	<b>0.6</b>	<b>0.0</b>	<b>0.5</b>	
PE	I	35.5		6.5	10.7	28.6	20.5	1.3	0.7
		±	0	±	±	±	±	±	±
	6.2		1.0	1.0	3.4	4.2	0.2	0.1	
	<b>34.8</b>		<b>4.2*</b>	<b>8.5*</b>	32.4	19.9	1.3	0.6	
TAG	M	±	0	±	±	±	±	±	±
		<b>0.3</b>		<b>0.0</b>	<b>0.0</b>	0.9	0.5	0.0	0.0
	41.4		5.9	4.0	36.7	11.8	1.1	0.3	
	±	0	±	±	±	±	±	±	
PG	I	10.0		0.6	0.6	7.3	3.4	0.2	0.0
		45.5		4.2	3.7	37.6	9.7	1.1	0.3
	±	0	±	±	±	±	±	±	
	<b>8.9</b>		<b>0.7</b>	<b>0.4</b>	<b>7.6</b>	<b>2.3</b>	<b>0.2</b>	<b>0.0</b>	
Other Lipids	M	27.9		16.1	13.4	26.8	15.9	1.1	0.6
		±	0	±	±	±	±	±	±
	0.1		2.5	2.9	0.9	4.4	0.1	0.1	
	<b>20.4*</b>		<b>10.1*</b>	10.1	<b>35.5*</b>	<b>26.5*</b>	1.5	0.8	
PA	I	±	0	±	±	±	±	±	±
		<b>1.8</b>		<b>1.3</b>	1.2	<b>2.6</b>	<b>3.8</b>	0.1	0.1
	36.5	33.9	4.0	19.5	4.4	4.7	0.4	1.1	
	±	±	±	±	±	±	±	±	
Free FA	M	2.2	1.4	1.6	1.2	1.1	1.9	0.1	0.2
		35.7	30.9	2.1	19.9	5.2	8.1	0.5	1.2
	±	±	±	±	±	±	±	±	
	<b>2.6</b>	<b>1.3</b>	<b>0.2</b>	<b>0.6</b>	<b>1.7</b>	<b>2.4</b>	<b>0.1</b>	<b>0.2</b>	
PI	I	28.4		21.9	10.3	23.1	16.3	1.1	0.7
		±	0	±	±	±	±	±	±
	2.0		1.1	2.0	0.0	1.2	0.1	0.1	
	<b>34.6</b>		<b>26.4*</b>	8.1	<b>18.2*</b>	<b>12.7*</b>	0.8	0.7	
Free FA	M	±	0	±	±	±	±	±	±
		<b>6.0</b>		<b>0.1</b>	2.6	<b>1.4</b>	<b>2.1</b>	0.1	0.1
	27.6		16.1	10.0	34.7	11.6	1.1	0.3	
	±	0	±	±	±	±	±	±	
Free FA	I	4.7		2.6	2.4	6.6	2.9	0.2	0.0
		32.8		12.1	6.6	34.4	14.9	1.2	0.4
	±	0	±	±	±	±	±	±	
	<b>5.0</b>		<b>1.1</b>	<b>1.8</b>	<b>4.4</b>	<b>2.8</b>	<b>0.1</b>	<b>0.1</b>	
Free FA	M	52.9		10.9	4.2	15.8	13.5	0.8	0.9
		±	0	±	±	±	±	±	±
	5.0		2.3	2.1	3.2	3.7	0.2	0.1	
	<b>59.2</b>		<b>8.0*</b>	3.0	19.7	13.1	0.8	0.7	
Free FA	I	±	0	±	±	±	±	±	±
		<b>7.7</b>		<b>1.2</b>	1.8	3.8	3.4	0.2	0.0
	35.3		56.5	3.5	2.8	2.0	0.2	0.7	
	±	0	±	±	±	±	±	±	
Free FA	M	0.9		0.0	0.0	0.5	0.5	0.0	0.0
		<b>41.0*</b>		<b>49.8*</b>	3.4	2.9	<b>3.0*</b>	0.2	<b>1.0*</b>
	±	0	±	±	±	±	±	±	
	<b>1.3</b>		<b>1.6</b>	0.0	0.3	<b>0.1</b>	0.0	<b>0.1</b>	

**Supplementary Table S2** - Fatty acids content in each lipid class of *V. vinifera* cv. Regent mock inoculated (M) and inoculated (I) leaves with *P. viticola*. Values correspond to average relative percentage ± standard error, n = 3; Asterisks indicate significant differences (p<0.05).

Family	Group	Proposed nomenclature	Locus	Nucleotide	Protein	Chr.	Introns	Protein length (aa)	Molecular weight (kDa)	pI	Domain (Blast2GO)	Subcellular location
DAD PLA <sub>1</sub>	I	VviPLA <sub>1</sub> -I $\alpha$	LOC100242894	XM_002268106.2	XP_002268142.1	5	0	446	49.8	7.04	Lipase 3	Chloroplast
		VviPLA <sub>1</sub> -I $\beta$ 1	LOC100242763	XM_002270956.4	XP_002270992.2	15	0	434	48.7	9.48	Lipase 3	Chloroplast
		VviPLA <sub>1</sub> -I $\beta$ 2	LOC100247003	XM_010648953.2	XP_010647255.1	unknown	0	528	59.1	9.04	Lipase 3	Chloroplast
		VviPLA <sub>1</sub> -I $\beta$ 3	LOC100259863	XM_002271029.4	XP_002271065.3	15	0	389	43.3	8.82	Lipase 3	-
		VviPLA <sub>1</sub> -I $\gamma$ 1	LOC100249474	XM_002281871.3	XP_002281907.1	7	1	514	58.2	7.07	Lipase 3	Chloroplast
	II	VviPLA <sub>1</sub> -I $\gamma$ 2	LOC100260417	XM_002285331.3	XP_002285367.1	6	0	502	57.7	8.59	Lipase 3	Chloroplast
		VviPLA <sub>1</sub> -II $\gamma$ 1a	LOC100242927	XM_002266946.3	XP_002266982.2	10	2	369	41.2	6.73	Lipase 3	Mitochondrial
		VviPLA <sub>1</sub> -II $\gamma$ 1b	LOC100242927	XM_019222491.1	XP_019078036.1	10	2	406	45.5	6.73	Lipase 3	Mitochondrial
		VviPLA <sub>1</sub> -II $\gamma$ 2	LOC100254098	XM_019223761.1	XP_019079306.1	12	3	403	45.7	8.93	Lipase 3	Mitochondrial
	III	VviPLA <sub>1</sub> -II $\delta$	LOC100267811	XM_002281059.4	XP_002281095.2	12	0	470	53.1	5.84	Lipase 3	Chloroplast
		VviPLA <sub>1</sub> -III $\alpha$ 1	LOC100266852	XM_002272744.3	XP_002272780.2	10	0	524	59.7	9.51	Lipase 3	-
		VviPLA <sub>1</sub> -III $\alpha$ 2	LOC100242997	XM_002272522.4	XP_002272558.2	10	0	511	58.8	7.32	Lipase 3	-
VviPLA <sub>1</sub> -III $\beta$ 1a		LOC100251427	XM_010657396.2	XP_010655698.1	10	0	510	58.0	7.07	Lipase 3	Chloroplast	
VviPLA <sub>1</sub> -III $\beta$ 1b		LOC100261664	XM_010657395.2	XP_010655697.1	10	0	510	58.1	7.72	Lipase 3	Chloroplast	
PA-preferring PLA <sub>1</sub>	VviPA-PLA <sub>1</sub> - $\alpha$	LOC100260107	XM_002275576.3	XP_002275612.1	18	20	971	109.4	5.03	DDHD	vacuole membrane / plasmodesma	
	VviPA-PLA <sub>1</sub> - $\beta$	LOC100260107	XM_010647575.2	XP_010645877.1	18	20	963	108.5	5.02	DDHD	vacuole membrane / plasmodesma	
Secretory PLA <sub>2</sub>	VvisPLA <sub>2</sub>	LOC100261227	XM_002282786.3	XP_002282822.1	11	3	151	16.3	8.70	-	integral component of membrane	
Patatin-like PLA	I	VvipPLA-I $\alpha$	LOC104880454	XM_010657059.2	XP_010655361.1	10	18	1067	118.5	5.88	Arm / Patatin domain	-
		VvipPLA-I $\beta$	LOC104880454	XM_019222598.1 XM_010657058.2	XP_019078143.1 XP_010655360.1	10	18	1316	146.2	5.83	Arm / Patatin domain	-
	II	VvipPLA-II $\alpha$ 1a	LOC100257463	XM_002282445.3	XP_002282481.1	18	6	413	45.0	5.97	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\alpha$ 1b	LOC109121419	XM_010666282.2	XP_010664584.1	18	6	413	45.1	5.97	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\alpha$ 2	LOC100266120	XM_002282561.3	XP_002282597.1	18	6	413	45.0	5.81	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\alpha$ 3	LOC100241962	XM_002284535.4	XP_002284571.1	18	6	425	46.5	5.28	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 1a	LOC100252227	XM_002282330.3	XP_002282366.2	18	6	411	45.4	6.35	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 1b	LOC100264244	XM_019216450.1	XP_019071995.1	18	6	411	45.3	6.35	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 2a	LOC100248840	XM_002282379.2	XP_002282415.2	18	6	406	44.9	6.98	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 2b	LOC100260810	XM_002282396.2	XP_002282432.1	18	7	406	44.9	6.98	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 3	LOC100255738	XM_002282404.1	XP_002282440.1	18	6	406	44.9	5.95	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 4a	LOC100254055	XM_019216314.1	XP_019071859.1	18	7	406	44.8	8.86	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\beta$ 4b	LOC100267749	XM_019216433.1	XP_019071978.1	18	6	406	44.7	8.85	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\gamma$ 1a	LOC100260531	XM_010653988.2	XP_010652290.1	7	12	425	47.0	6.27	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\gamma$ 1b	LOC100264086	XM_002272007.3	XP_002272043.1	7	6	426	47.1	6.33	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\gamma$ 2	LOC100260531	XM_002271666.3	XP_002271702.1	7	12	424	46.6	9.21	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\delta$ 1	LOC100249295	XM_002281762.4	XP_002281798.1	7	6	407	44.0	5.31	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\delta$ 2	LOC100245674	XM_002277282.3	XP_002277318.1	7	6	402	44.2	5.48	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\delta$ 3	LOC100250815	XM_002277269.2	XP_002277305.1	7	7	407	44.5	5.86	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\epsilon$ 1	LOC100244172	XM_002262906.3	XP_002262942.1	unknown	5	397	43.6	5.96	Patatin domain	Cytoplasm / membrane
		VvipPLA-II $\epsilon$ 2	LOC100262840	XM_019221012.1	XP_019076557.1	7	6	398	44.1	7.16	Patatin domain	Cytoplasm / membrane
		III	VvipPLA-III $\alpha$	LOC100252820	XM_002276301.3	XP_002276337.2	10	1	484	52.4	9.18	Patatin domain
	VvipPLA-III $\beta$		LOC100246118	XM_010660108.2 XM_019224348.1	XP_010658410.1 XP_019079893.1	13	4	458	49.7	6.91	Patatin domain	-
	VvipPLA-III $\delta$		LOC100263127	XM_002277845.4	XP_002277881.1	7	1	385	42.0	8.85	Patatin domain	-

**Supplementary Table S3** - General features of grapevine PLA superfamily. Proposed grapevine PLA nomenclature, gene locus, protein and nucleotide accessions (from NCBI and CRIBI), chromosome locations, intron number, protein length, molecular weight, isoelectric point (pI), domain and subcellular prediction are represented.

PLA gene	Nucleotide	PLA Location (Mb)	Chr.	<i>Rpv</i>	RPV Location (Mb)	Associated marker	References
<i>VviPLA1-Iα</i>	XM_002268106.2	20.6	5	<i>Rpv14</i>		GF05-13	Ochssner et al. 2016
<i>VviPLA1-Iα2</i>	XM_002281871.3	0.2					
<i>VviPLA1-Iγ1</i>	XM_002277845.4	1.3		<i>Rpv7</i>	11.4	UDV-097	Bellin et al. 2009
<b><i>VvipPLA-IIδ3*</i></b>	XM_002277269.2	17					
<b><i>VvipPLA-IIδ2*</i></b>	XM_002277282.3	17	7				
<b><i>VvipPLA-IIδ1*</i></b>	XM_002281762.4	17		<i>Rpv9</i>	16.6	CCoAOMT	Moreira et al. 2011
<i>VvipPLA-IIγ1a</i>	XM_010653988.2	4.3					
<i>VvipPLA-IIγ1b</i>	XM_002272007.3	4.3					
<i>VviPLA1-IIδ</i>	XM_002281059.4	2.6		<i>Rpv1</i>	10.3	VVIb32	Merdinoglu et al. 2003
			12	<i>Rpv6</i>	20.4	VMC8G9	Marguerit et al. 2009
<i>VviPLA1-IIγ2</i>	XM_019223761.1	3.1		<i>Rpv13</i>	10.0	VMC1G3.2	Moreira et al. 2011
<i>VviPA-PLA1-α</i>	XM_002275576.3	0.9					
<i>VviPA-PLA1-β</i>	XM_010647575.2	0.9					
<i>VvipPLA-IIα3</i>	XM_002284535.4	9.1		<i>Rpv2</i>			Wiedemann-Merdinoglu et al. 2006; Bellin et al. 2009
<i>VvipPLA-IIα2</i>	XM_002282561.3	9.1					
<i>VvipPLA-IIα1b</i>	XM_010666282.2	9.1					
<i>VvipPLA-IIβ4a*</i>	XM_019216314.1	9.1					
<i>VvipPLA-IIα1a</i>	XM_002282445.3	9.2	18	<i>Rpv3</i>	24.9 26.9	UDV-112 UDV-305 VMC7f2	Welter et al. 2007; Bellin et al. 2009
<i>VvipPLA-IIβ3</i>	XM_002282404.1	9.2					
<i>VvipPLA-IIβ2b</i>	XM_002282396.2	9.2					
<i>VvipPLA-IIβ2a</i>	XM_002282379.2	9.2					
<i>VvipPLA-IIβ1b</i>	XM_019216450.1	9.3		<i>Rpv15</i>			Pap et al. (in preparation)
<i>VvipPLA-IIβ1a</i>	XM_002282330.3	9.3					

**Supplementary Table S4** - Grapevine PLA genes and chromosomal location of “Resistance to *Plasmopara viticola* (*Rpv*)” loci. Bold indicate PLA selected for further studies, bold and asterisks indicate the PLA with putative involvement in defence.

Gene name	Abbreviation	NCBI Accession Number	Primer Sequence	Amplicon length (bp)	Ta (°C)	Tm (°C)	Amplification efficiency (E)	
<b>Elongation Factor 1-alpha</b>	<i>EF1α</i>	XM_002284888.2	Fw: GAACTGGGTGCTTGATAGGC Rev: ACCAAAATATCCGGAGTAAAAGA	149	60	79.45	1.89	
<b>Ubiquitin-conjugating enzyme</b>	<i>UBQ</i>	XM_002284161.3	Fw: GAGGGTCGTCAGGATTTGGA Rev: GCCCTGCACTTACCATCTTTAAG	75	60	78.65	1.97	
<b>Phospholipase A1</b>	<b>Group I</b>	<i>VviPLA1-Iβ1</i>	XM_002270956.4	Fw: TCCCTAGTGCTCATTCCGAT Rev: TTGAAGAAGACTGGCGACCT	122	58	78.81	2.05
		<i>VviPLA1-Iγ1</i>	XM_002281871.3	Fw: ACTGCGGGACCTGTAGATTT Rev: TTAGCCATCGGGACTTCTT	142	60	80.53	2.00
	<b>Group II</b>	<i>VviPLA1-IIδ</i>	XM_002281059.4	Fw: ATGAGGAGGGGAGTAGCAG Rev: TCTGGGGTCACCTGAAGTG	104	60	77.17	1.94
<b>Secretory Phospholipase A2</b>	<i>VvisPLA2</i>	XM_002282786.3	FW: CAGGTGCGCCAATAGGACA Rev: CGGCGACTGCGAAGAAGAA	89	60	81.71	1.91	
<b>Patatin-like phospholipase A</b>	<b>Group I</b>	<i>VvipPLA-Iα*</i>	XM_010657059.2	FW: TTGTGCCTGAACCCCGTGT Rev: CCTTTCTTCCCAACCTGTCT	109	60	80.48	2.06
	<b>Group II</b>	<i>VvipPLA-IIα1/2*</i>	XM_002282445.3	FW: TTCATACCTCCATCGCTCC Rev: CCTCTTATTCCACCTCCAT	Not detected due to the low presence of transcript			
		<i>VvipPLA-IIβ*</i>	XM_019216314.1	FW: CTGCAGCACCAACATACTTT Rev: CTGCGACACCACCATCAATA	96	58	75.59	1.90
		<i>VvipPLA-IIδ1</i>	XM_002281762.4	FW: ATAACAACGGGTCCACTCCA Rev: ATCCGAGGGCCTGAAATACA	Not detected due to the low presence of transcript			
		<i>VvipPLA-IIδ2</i>	XM_002277282.3	FW: GACTCCGTGAGTCAATGGC Rev: TGGGGTGAAGAAGTGGTCAA	116	60	75.57	2.02
		<i>VvipPLA-IIδ3</i>	XM_002277269.2	FW: CGGAATCAAGCCTTTTACC Rev: TCATATTTTGGCCCTGTTAG	Not detected due to the low presence of transcript			
	<b>Group III</b>	<i>VvipPLA-IIIβ</i>	XM_010660108.2 XM_019224348.1	FW: ACGTTGAAGCCGGTGTGAT Rev: GGAAGTCGAAGCTGTCTGTT	100	60	82.6	1.92

**Supplementary Table S5** - Reference and target genes transcripts primer sequences, amplicon length, amplification efficiency, annealing and melting temperature are represented.