Myrigalone A inhibits *Lepidium sativum* seed germination by interference with gibberellin metabolism and apoplastic superoxide production required for embryo extension growth and endosperm rupture

Krystyna Oracz[1,*], Antje Voegele[1], Danuše Tarkowská[2], Dominique Jacquemoud[1], Veronika Turečková[2], Terezie Urbanová[2], Miroslav Strnad[2,3], Elwira Sliwinska[4] and Gerhard Leubner-Metzger[1,*]

[1] University of Freiburg, Faculty of Biology, Institute for Biology II, Botany / Plant Physiology, D-79104 Freiburg, Germany, Web: 'The Seed Biology Place' - www.seedbiology.de

[2] Laboratory of Growth Regulators, Faculty of Science, Palacky University and Institute of Experimental Botany AS CR, v.v.i., Šlechtitelů 11, CZ-783 71, Olomouc, Czech Republic

[3] Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacky University, Šlechtitelů 11, CZ-783 71, Olomouc, Czech Republic

[4] Laboratory of Molecular Biology and Cytometry, Department of Plant Genetics and Biotechnology, University of Technology and Life Sciences, Kaliskiego Ave. 7, PL-85-789 Bydgoszcz, Poland

* Joint corresponding authors: Email, oracz.krystyna@op.pl and gerhard.leubner@biologie.uni-freiburg.de

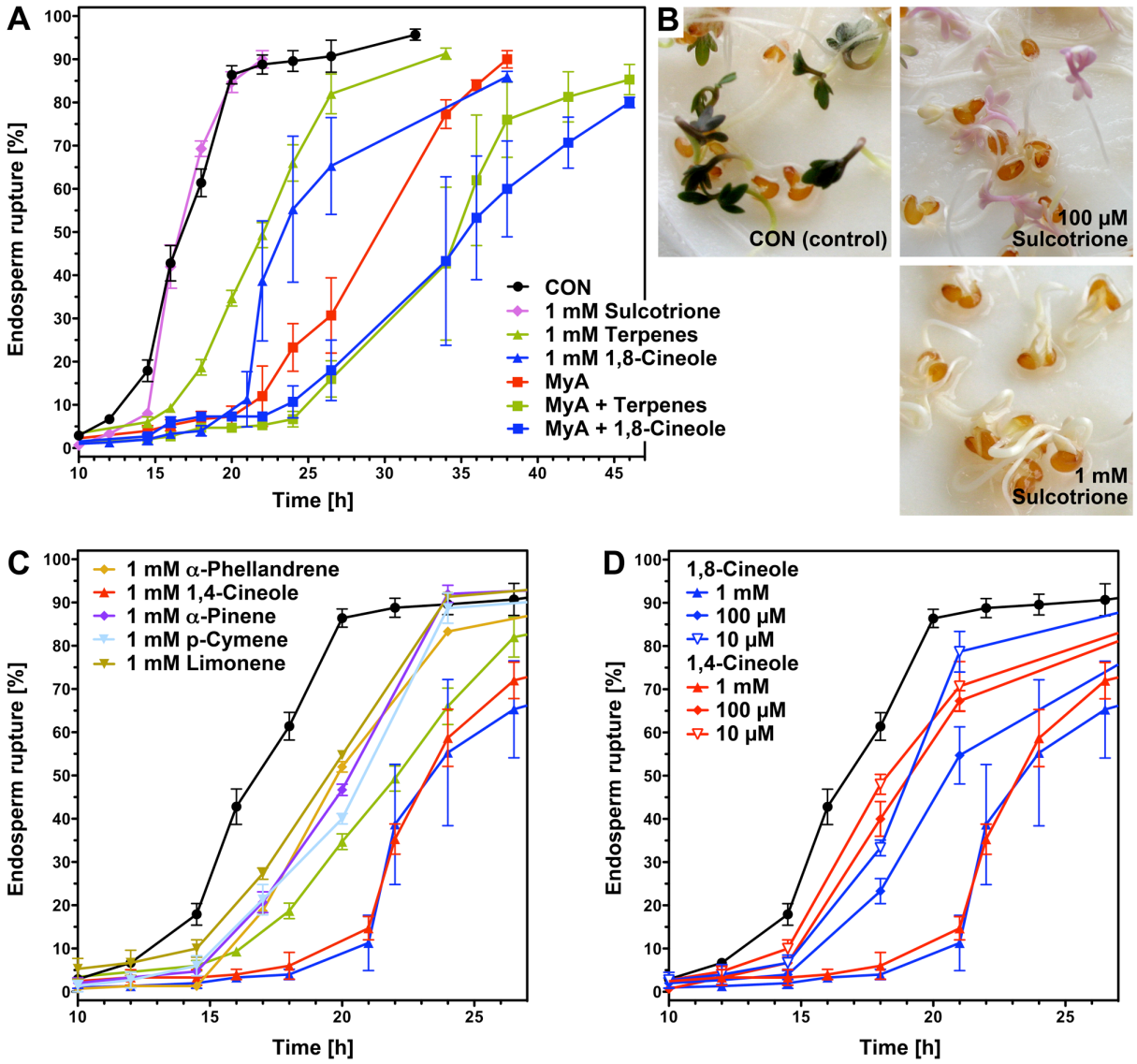
Phone ++49 761 203 2669 and ++49 761 203 2936, Fax ++49 761 203 2612

Plant and Cell Physiology

Special Issue on Seed Biology (Issue 1, 2012)

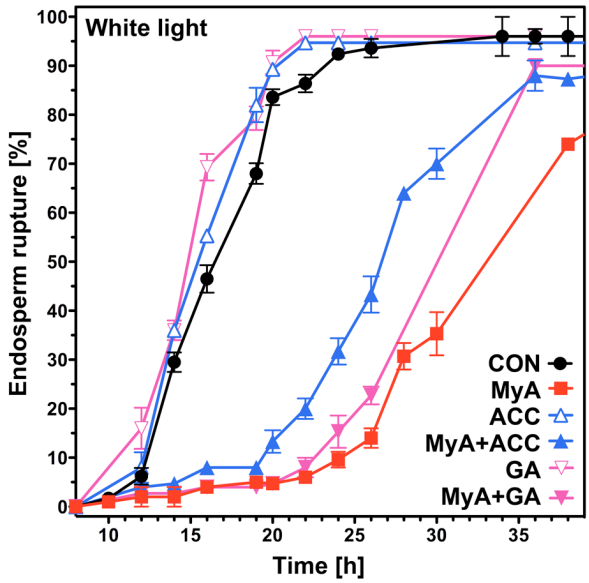
Supplementary data

Supplementary Figure S1



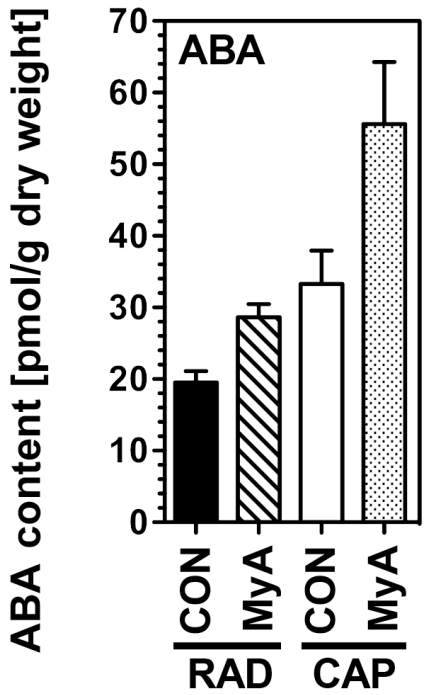
Supplementary Fig. S1 The effects of MyA, sulcotrione (commercial herbicide) and different terpenes on the endosperm rupture of germinating *Lepidium sativum* seeds in continuous white light. (A) Effect of terpenes (1 mM terpenes is a mixture of all the terpenes indicated in the figure) and sulcotrione. (B) Seedlings grown from seeds germinated in the presence of the herbicide sulcotrione. (C) The effect of various terpenes present in exudates of *Myrica gale* fruits and leaves on the endosperm rupture. (D) Endosperm rupture dose response of *L. sativum* seeds incubated in the presence of 1,4-and 1,8-cineole. CON = control without terpene, sulcotrione or MyA addition; MyA = 5 x 10^{-4} MyA; Terpenes = mixture of α-pinene, 1,8-cineole, *p*-cymene, α-phellandrene, limonene, each in 1-mM concentration.

Supplementary Figure S2



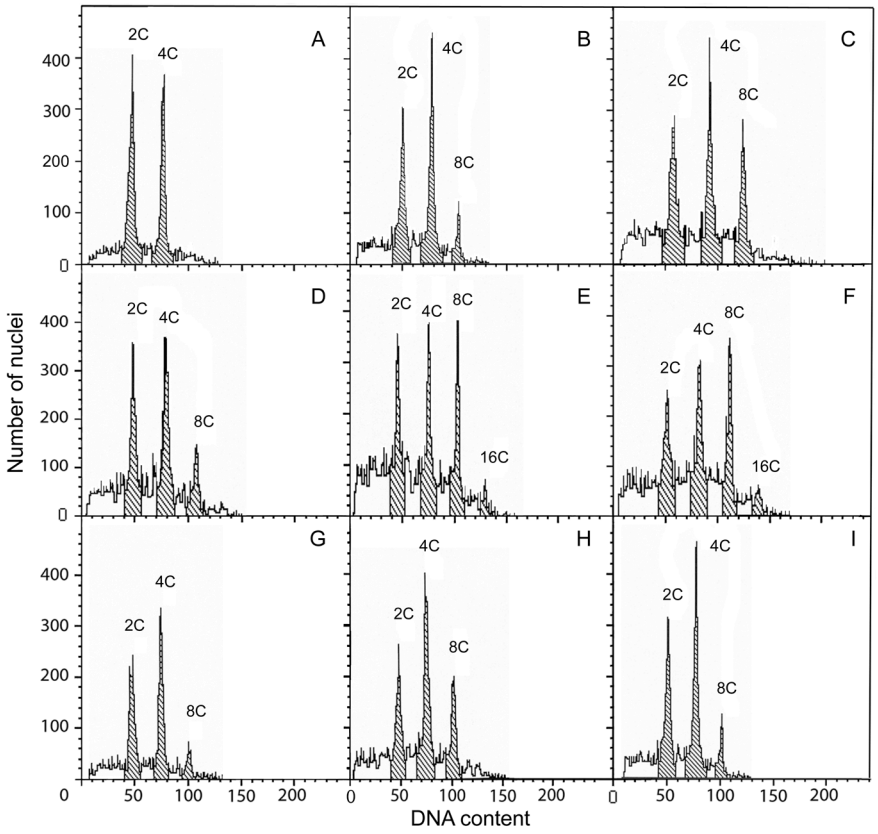
Supplementary Fig. S2 The effect of myrigalone A (MyA) and plant hormones on the endosperm rupture of *Lepidium sativum* seeds imbibed in continuous white light. 1 mM ACC and 10 µM GA_{4+7} (GA) were applied alone or combined with $5x10^{-4}$ M MyA. CON = control without GA, ACC or MyA addition.

Supplementary Figure S3



Supplementary Fig. S3 The tissue-specific (RAD, CAP) effect of myrigalone A (MyA) on abscisic acid (ABA) contents during the germination of *Lepidium sativum* seeds incubated in continuous white light. ABA contents in RAD and CAP isolated from CON and $5x10^{-4}$ M MyA-treated seeds imbibed for 15 h were quantified. CON = control without MyA addition.

Supplementary Figure S4



Supplementary Fig. S4 Selected DNA histograms (flow cytometry) of nuclear preparations from RAD zones isolated from dry *Lepidium sativum* seeds (A, D, G), control (B, E, H) and MyA ($5x10^{-4}$ M My) treated (C, F, I) seeds imbibed for 22 h in continuous white light. Results obtained from Zone 1 of RAD are presented on histograms A, B, C; from Zone 2 on histograms D, E, F; and from Zone 3 on histograms G, H, I. The localization of three distinct zones is shown on the **Fig. 6** and the quantification is in **Supplemental Table S2**.

Supplementary Table S1 Contents of active and inactive gibberellins (GA) in RAD and CAP isolated from *Lepidium sativum* seeds imbibed for 15 h without (CON) or with $5x10^{-4}$ M MyA added in continuous white light. The two major GA metabolic pathways, 13-non-hydroxylated and 13-hydroxylated, are indicated. Means ± SE of 4 biological replicates are presented and expressed in pg/mg dry weight.

		RAD			CAP	
		CON	MyA		CON	MyA
13-Non-hydroxylation pathway						
GA_{12ald}		5.3 ± 2.2	0.0 ± 0.0		27.5 ± 16.1	0.0 ± 0.0
GA_{12}		3.7 ± 0.2	5.7 ± 0.8		0.0 ± 0.0	3.8 ± 3.8
GA_{15}		6.0 ± 2.4	6.7 ± 1.4		4.5 ± 4.5	16.9 ± 8.4
GA_{24}		45.0 ± 1.8	36.2 ± 11.5		32.2 ± 9.5	45.1 ± 13.9
GA_{9}		26.5 ± 3.5	5160 ± 652		13.9 ± 6.5	6450 ± 4016
GA_{51}		4.2 ± 3.3	5.4 ± 3.7		44.5 ± 5.4	0.0 ± 0.0
GA_{4}		56.8 ± 8.7	23.4 ± 2.9		26.4 ± 11.3	33.8 ± 22.9
GA_{34}		2.3 ± 0.1	3.6 ± 0.8		4.6 ± 1.2	5.1 ± 0.9
GA_{13}		6.2 ± 0.9	2.6 ± 0.9		53.4 ± 6.2	55.5 ± 13.8
13-Hydroxylation pathway						
GA_{53}		17.3 ± 4.3	4.8 ± 2.1		78.8 ± 10.2	30.0 ± 9.6
GA_{44}		3.8 ± 2.2	3.6 ± 3.6		9.8 ± 6.1	8.5 ± 8.5
GA_{19}		3.9 ± 2.4	2.6 ± 1.1		0.0 ± 0.0	0.0 ± 0.0
GA_{20}		4.9 ± 2.2	1.6 ± 1.6		0.0 ± 0.0	0.0 ± 0.0
GA_{29}		9.5 ± 3.2	10.8 ± 2.9		75.8 ± 13.7	17.0 ± 5.8
GA_{1}		5.4 ± 1.2	4.4 ± 1.6		2.7 ± 2.7	0.0 ± 0.0
GA_{8}		60.2 ± 2.7	50.2 ± 6.8		30.5 ± 20.9	18.6 ± 7.0
GA_{5}		0.9 ± 0.9	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0
GA_{6}		55.8 ± 4.8	36.6 ± 7.9		412.2 ± 41.3	20.8 ± 9.1
GA_{3}		1.2 ± 0.1	0.9 ± 0.2		0.0 ± 0.0	0.4 ± 0.4
GA_{7}		0.0 ± 0.0	0.0 ± 0.0		0.0 ± 0.0	0.0 ± 0.0

Supplementary Table S2. Effect of MyA ($5x10^{-4}$ M) on endoreduplication of nuclei (flow cytometry) in different zones of the *Lepidium sativum* hypocotyl/radicle-region (RAD) of embryos isolated from seeds imbibed in continuous white light (see Fig. 6 for details).

Treatment	Time of imbibition (h)	Percentage of nuclei with DNA content				Mean C-value (± SD)		(4C+8C+16C)/2C ratio (± SD)	
		2C	4C	8C	16C				
Zone 1									
Control	0	57.0	43.0	0	0	2.86 ± 0.14	d*	0.78 ± 0.23	e
	15	35.0	53.5	11.5	0	3.76 ± 0.16	bc	1.87 ± 0.22	bc
	22	34.1	49.7	16.2	0	3.97 ± 0.46	b	2.02 ± 0.60	b
	30	19.5	46.4	23.9	10.2	5.80 ± 0.42	a	4.29 ± 0.34	a
MyA	15	43.4	47.8	8.8	0	3.48 ± 0.22	c	1.32 ± 0.25	d
	22	41.0	49.5	9.5	0	3.56 ± 0.07	c	1.44 ± 0.10	cd
	30	42.5	47.5	10.0	0	3.55 ± 0.16	c	1.39 ± 0.34	d
Zone 2									
Control	0	43.8	47.1	9.1	0	3.49 ± 0.31	e	1.31 ± 0.29	d
	15	30.3	43.8	25.9	0	4.43 ± 0.11	cd	2.32 ± 0.33	bc
	22	27.8	39.7	27.7	4.8	5.13 ± 0.32	b	2.69 ± 0.66	b
	30	17.3	36.5	32.1	14.1	6.63 ± 0.62	a	5.31 ± 2.13	a
MyA	15	35.2	43.5	21.3	0	4.15 ± 0.21	d	1.86 ± 0.26	cd
	22	34.3	41.8	23.9	0	4.27 ± 0.09	d	1.94 ± 0.32	bc
	30	32.0	38.9	27.2	1.9	4.68 ± 0.31	c	2.14 ± 0.30	bc
Zone 3									
Control	0	38.6	39.3	22.1	0	4.11 ± 0.25	c	1.61 ± 0.27	c
	15	35.5	41.0	23.5	0	4.27 ± 0.11	c	1.89 ± 0.40	bc
	22	31.6	37.3	27.9	3.2	4.86 ± 0.45	b	2.19 ± 0.31	b
	30	22.1	38.7	33.4	5.8	5.59 ± 0.70	a	3.75 ± 1.15	a
MyA	15	34.3	39.6	26.1	0	4.36 ± 0.16	c	1.92 ± 0.13	bc
	22	33.5	41.8	24.7	0	4.32 ± 0.26	c	2.02 ± 0.41	bc
	30	34.2	42.6	23.2	0	4.24 ± 0.06	c	1.93 ± 0.09	bc

*values for a certain parameter and axis zone followed by the same letters are not significantly different at P=0.05 (Duncan's test)

Supplementary Table S3 Chemicals and concentrations used in germination assays with *Lepidium sativum* seeds.

Treatment	Final concentration	Origin (company, cat. no)
Myrigalone A (MyA)[a]	0.1 µM to 1 mM	Prof. Gilles Comte, CESN, Lyon, France
Sulcotrione[b] (synthetic herbicide)	10 nM to 1 mM	Fluka, cat. no 46318
Gibberellin A_{4+7} (GA)	10 µM	Duchefa, cat. no G0938
1-aminocyclopropane-1-carboxylic-acid (ACC)	1 mM	Sigma, cat. no A3903
cis-*S*(+)-abscisic acid (ABA)	3 µM	Duchefa, cat. no A0941
α-pinene	1 mM	Aldrich, cat. no 14752-4
α-phellandrene	1 mM	Aldrich, cat. no W28560
p-cymene	1 mM	Aldrich, cat. no C12145-2
1,8-cineol	1 mM	Aldrich, cat. no C80601
1,4-cineol[b]	1 mM	Fluka, cat. no 27393
Limonene[c]	1 mM	Fluka, cat. no 62118

[a] >99% purity; dissolved as a stock in methanol; therefore as control (CON) 0.35 (v/v) methanol was used in germination assays with *L. sativum* seeds.
[b] Not present in *M. gale* fruits or leaves.
[c] Identified in *M. gale* leaves, but not in fruit exudates.

Supplementary Table S4 Main characteristics of genes and primer sequences for qRT-PCR used in the present work.

Abbreviation of gene	Description of gene	Specificity of primer	Accession number	Forward Primer: sequence 5' --> 3' Reverse Primer: sequence 5' --> 3'
SDR1/ABA2	cytosolic short-chain dehydrogenase/reductase	*A. thaliana*	At1g52340	F : ATGTGGAGCACCGTGCCCTG R : AACGCCTCCCACAACACCTCC
LesaCYP707A2	ABA 8'-hydroxylase CYP707A2	*L. sativum*	GQ221028 (At2g29090)	F : AAGAGCTTTCATGCCGGATTC R : AAGAGCTTTCATGCCGGATTC
LesaCYP707A3	ABA 8'-hydroxylase CYP707A3	*L. sativum*	GQ221029 (At5g45340)	F : ATCAACACCCTCGAACACATG R : TCAATTTCAGTGGCCTCCTCTT
LesaGID1a	GA receptor GIBBERLLIN INSENSITIVE DWARF 1a	*L. sativum*	HQ003455 (At3g05120)	F: AGCGGGTGAGTCTGGAATCAAT R: AAGTTTCTCAGACTCTGTTCTC
LesaGID1b	GA receptor GIBBERLLIN INSENSITIVE DWARF 1b	*L. sativum*	HQ003456 (At3g63010)	F: AGCTCACAATGTTGCTGTGAG R: TCAGTCCTCTCTTGTCCAC
LesaGID1c	GA receptor GIBBERLLIN INSENSITIVE DWARF 1c	*L. sativum*	HQ003457 (At5g27320)	F: GCATAATGTCGCGGTTAGAG R: GTCCCTCCAAACATAGGGTT
GA3ox1	GA3 oxidase 1	*A. thaliana*	At1g15550	F: TGGGCCCAAGCTGCTCTCCA R: AGCCAGGAAACGGTGGCACG
GA3ox2	GA3 oxidase 2	*A. thaliana*	At1g80340	F: GATGGACGTGGGCTGGGTTA R: CCCTGGCGGTGAAGCACG
GA3ox3	GA3 oxidase 3	*A. thaliana*	At4g21690	F: AACCCCGTGAACCGTGACCG R: ACGGGAATGGGTCCGCTCGT
GA3ox4	GA3 oxidase 4	*A. thaliana*	At1g80330	F: GTGGGCAGAGGCGCCATACG R: CCGGCTCAACCGTGACCCAA
GA2ox7	GA2 oxidase7	*A. thaliana*	At1g50960	F: GCAGCTGCGAAAGAGTGGGGA R: TGAGCGGGAGAAGTGGCGCT
Lesa17210	IPL1; IAP-like protein 1, zinc ion binding	*L. sativum*	HQ912755 (At1g17210)	F: TCCGCCCTTGTATGGACGAGA R: CTCTGGCACCTGGGAAAGCCA
Lesa20000	HBT; anaphase-promoting complex subunit	*L. sativum*	HQ912757 (At2g20000)	F: TCTGGTCCACGACGGAGCTTG R: TCTGGTCCACGACGGAGCTTG
Lesa04660	APC2; ubiquitin-protein ligase cell cycle regulation	*L. sativum*	HQ912754 (At2g04660)	F: AGCTGGGTCTATTGCACGAAG R: AGCTGGGTCTATTGCACGAAG