Embryo growth, testa permeability and endosperm weakening are major targets for the environmentally regulated inhibition of *Lepidium sativum* seed germination by myrigalone A

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Supplementary Material

Figure S1

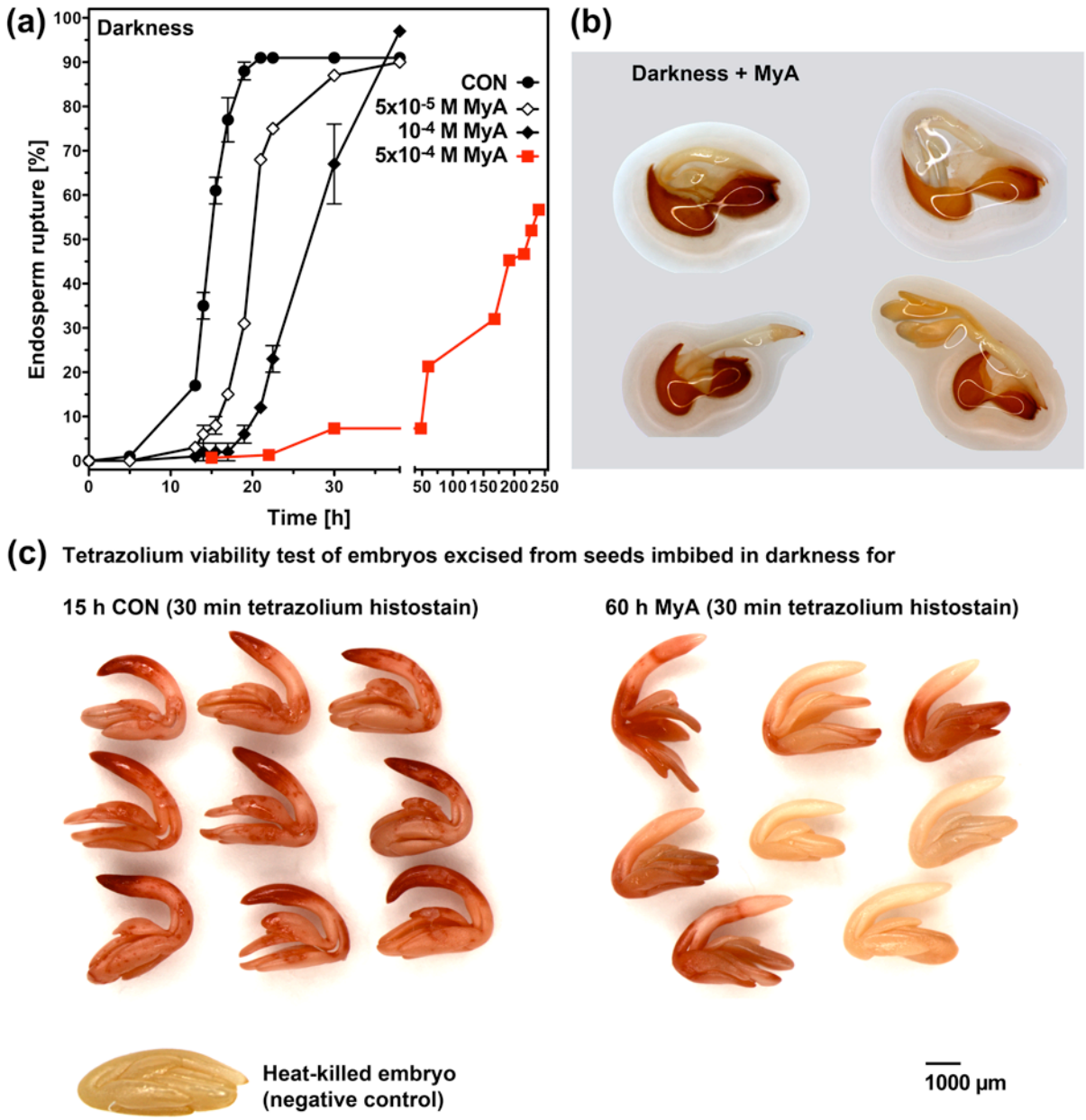

Figure S1. The effect of myrigalone A (MyA) on the germination of *Lepidium sativum* seeds imbibed in darkness. (a) Germination kinetics of CON and seeds treated with different MyA concentrations. The times to 50% endosperm rupture are 14.9±0.1 h, 20.0±0.0 h, 27.2±1.1 h, and 223.0±2.3 h for CON, $5x10^{-5}$ M MyA, 10^{-4} M MyA, and $5x10^{-4}$ M MyA, respectively. (b) Atypical germination of seeds incubated in the presence of MyA in darkness. (c) Tetrazolium viability assay of embryos from seeds incubated in darkness. Embryos were excised from seeds prior to endosperm rupture and incubated in tetrazolium solution for 30 min. CON = 0.35% (v/v) methanol.

Figure S2

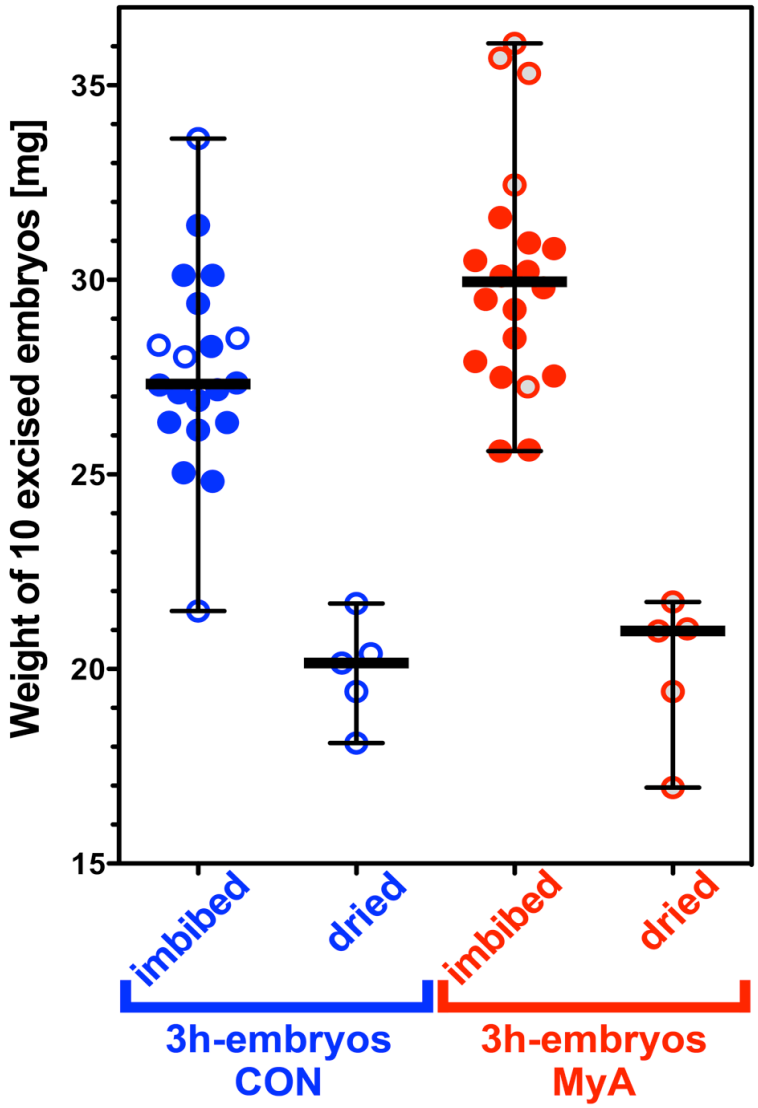

Figure S2. The effect of myrigalone A (MyA) on embryo water uptake by imbibition during the early phase of *Lepidium sativum* seeds imbibed for 3h in continuous light without (CON) or with $5x10^{-4}$ M MyA added. The weights of 10 embryos per data point excised from 3h-imbibed seeds were compared before and after over-night air-drying; the black lines indicate the median ± the data range. A *t*-test was carried out using GraphPad Prism 5.03 for determination of significance. Imbibed MyA-treated embryos are ca. 1.1-fold heavier compared to imbibed CON-embryos (P = 0.0098), while the weights of air-dried embryos did not differ (P = 0.9461).

Figure S3

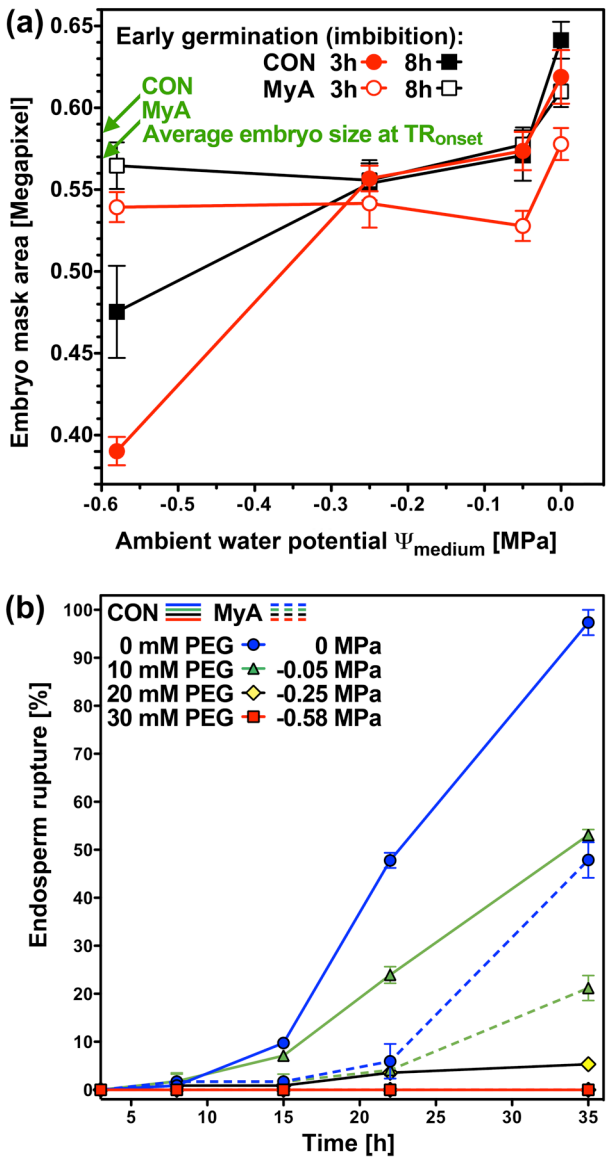

Figure S3. The effect of MyA and the ambient water potential (Ψ_{medium}) on embryo imbibition, embryo size and endosperm rupture during the germination of *L. sativum* seeds incubated in continuous white light.

(a) Early germination: Embryo mask areas at different Ψ_{medium} in the CON- and MyA-series during the early (3h and 8h) phase of germination. The early phase is characterized by MyA-enhanced imbibition (phase-I water uptake). Embryo sizes at the onset of testa rupture (TR) are indicated.

(b) Late germination: Time courses of endosperm rupture of seed populations incubated without (CON) or with $5x10^{-4}$ M MyA, and 0, 10, 20 or 30 mM PEG as osmoticum; Ψ_{medium} of the different PEG concentrations are indicated.

Figure S4

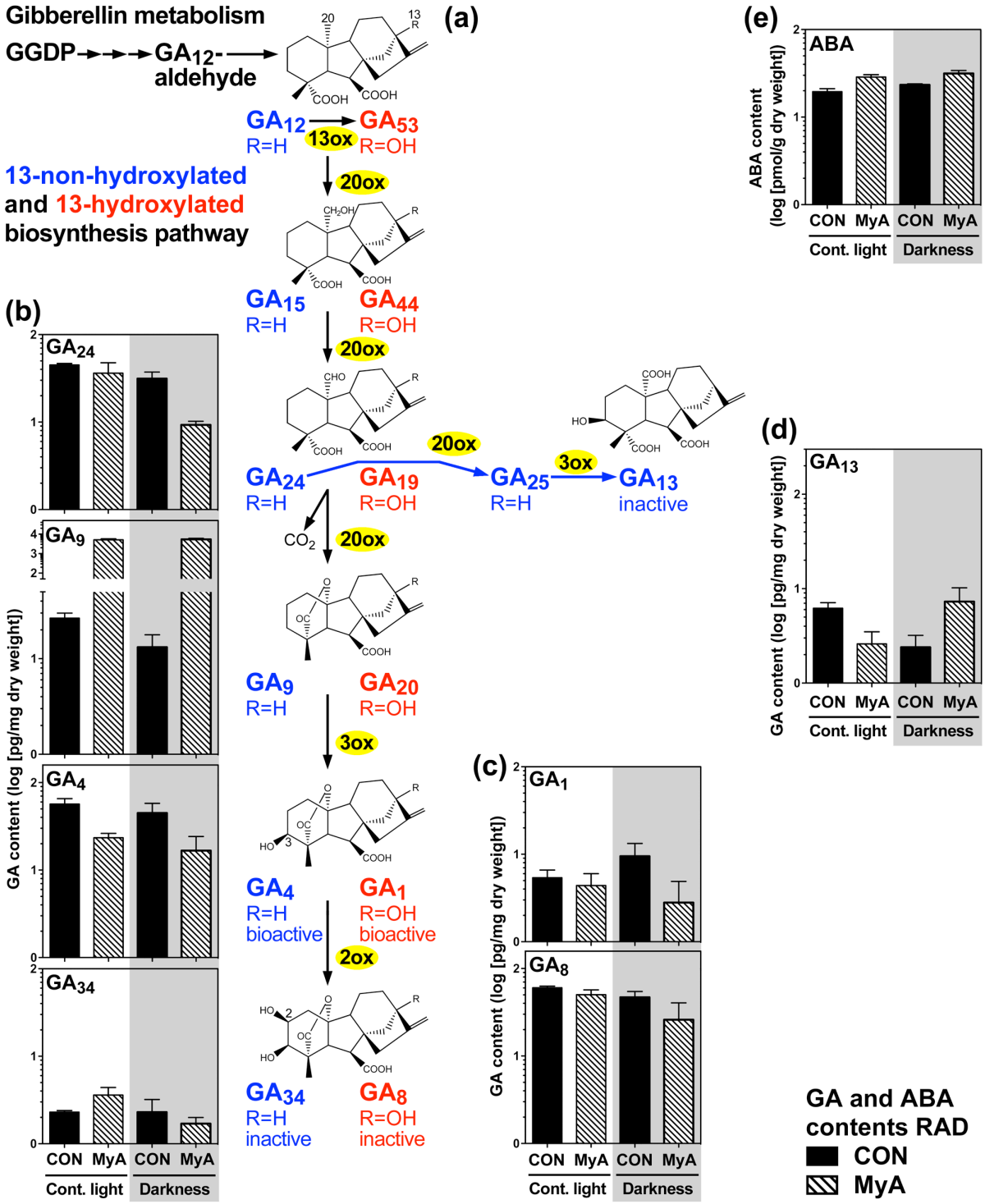

Figure S4. The tissue-specific effect of MyA on gibberellin (GA) and ABA metabolism during the germination of *L. sativum* seeds incubated in continuous light or darkness without (CON) or with $5x10^{-4}$ M MyA added.

(a) The 13-non-hydroxylated (blue) and the 13-hydroxylated (red) GA biosynthesis and inactivation pathways and important metabolites detected in *L. sativum* seeds. (b-d) Contents of bioactive gibberellins such as GA_1 and GA_4, and inactive forms such as GA_{24}, GA_9, GA_{34}, GA_8, GA_{13} and of ABA (e) quantified in RAD extracted from CON and MyA-treated seeds incubated for 15h. For comparison to darkness (this work), values for continuous light (Oracz *et al.* 2012) are presented. Note that metabolite contents are expressed in log scale as mean values ± SE of four biological replicates.

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

	Continuous white light				Darkness			
	RAD		CAP		RAD		CAP	
	CON	MyA	CON	MyA	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	13.8 ± 1.2	17.4 ± 13.7	187.4 ± 69.9	15.5 ± 15.5
GA_{12}	3.7 ± 0.2	5.7 ± 0.8	0.0 ± 0.0	3.8 ± 3.8	0.7 ± 0.7	0.0 ± 0.0	5.4 ± 5.4	4.3 ± 4.3
GA_{15}	6.0 ± 2.4	6.7 ± 1.4	4.5 ± 4.5	16.9 ± 8.4	5.9 ± 1.2	1.1 ± 1.1	10.2 ± 6.4	21.2 ± 7.6
GA_{24}	45.0 ± 1.8	36.2 ± 11.5	32.2 ± 9.5	45.1 ± 13.9	31.4 ± 5.9	9.3 ± 0.9	31.5 ± 10.8	52.6 ± 3.2
GA_{9}	26.5 ± 3.5	5160 ± 652	13.9 ± 6.5	6450 ± 4016	13.2 ± 4.6	5464 ± 631	11.4 ± 0.9	25820 ± 3062
GA_{51}	4.2 ± 3.3	5.4 ± 3.7	44.5 ± 5.4	0.0 ± 0.0	4.8 ± 4.8	4.9 ± 4.9	9.3 ± 5.4	27.2 ± 27.2
GA_{4}	56.8 ± 8.7	23.4 ± 2.9	26.4 ± 11.3	33.8 ± 22.9	45.0 ± 12.9	16.7 ± 7.5	42.9 ± 13.3	42.3 ± 6.0
GA_{34}	2.3 ± 0.1	3.6 ± 0.8	4.6 ± 1.2	5.1 ± 0.9	2.3 ± 0.9	1.7 ± 0.3	16.9 ± 13.1	7.2 ± 2.6
GA_{13}	6.2 ± 0.9	2.6 ± 0.9	53.4 ± 6.2	55.5 ± 13.8	2.4 ± 0.8	7.3 ± 2.9	89.9 ± 27.2	28.8 ± 14.3
13-Hydroxylation pathway								
GA_{53}	17.3 ± 4.3	4.8 ± 2.1	78.8 ± 10.2	30.0 ± 9.6	10.1 ± 2.1	11.9 ± 2.8	46.6 ± 16.8	38.1 ± 6.9
GA_{44}	3.8 ± 2.2	3.6 ± 3.6	9.8 ± 6.1	8.5 ± 8.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GA_{19}	3.9 ± 2.4	2.6 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	2.3 ± 1.3	2.7 ± 1.6	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	0.0 ± 0.0	0.0 ± 0.0	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	4.3 ± 2.0	13.4 ± 6.4	47.4 ± 20.7	55.3 ± 25.5
GA_{1}	5.4 ± 1.2	4.4 ± 1.6	2.7 ± 2.7	0.0 ± 0.0	9.5 ± 3.8	2.8 ± 2.1	0.0 ± 0.0	0.0 ± 0.0
GA_{8}	60.2 ± 2.7	50.2 ± 6.8	30.5 ± 20.9	18.6 ± 7.0	46.9 ± 7.8	26.0 ± 14.5	36.2 ± 5.6	41.7 ± 36.0
GA_{5}	0.9 ± 0.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
GA_{3}	1.2 ± 0.1	0.9 ± 0.2	0.0 ± 0.0	0.4 ± 0.4	0.1 ± 0.1	1.4 ± 1.3	1.8 ± 1.2	1.6 ± 0.6
GA_{7}	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Abscisic acid (ABA) and ABA-glucoseester (ABA-GE)								
ABA	19.5 ± 1.6	28.7 ± 1.8	33.3 ± 4.7	55.6 ± 8.7	23.4 ± 0.6	31.5 ± 2.6	61.6 ± 3.8	45.9 ± 2.0
ABA-GE	201.2 ± 15.4	174.3 ± 27.5	129.5 ± 15.4	144.6 ± 35.0	215.7 ± 35.3	209.1 ± 37.7	172.4 ± 22.7	169.5 ± 29.1