

Supplementary Materials

***Salvia* species: biotechnological strategies applied on *in vitro* cultures for the controlled production of bioactive diterpenoids**

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Table S1. Effect of microorganism interaction and their extracts on diterpenoids production in *in vitro* *Salvia* hairy roots cultures.

<i>Salvia</i> <i>sp.</i>	<i>A.</i> <i>Rhizog</i>	Culture medium	Growth condition	Elicitors	Conc. elicitor	Of on day	Elicited elicitatio	Time n (days)	Biomass growth vs CTRL	Diterpenes	Yield of control	Yield after elicitation	% Increase	Refere nces
<i>S. miltiorrhiza</i>	ATCC 15834	MS medium	0.3 g fresh root in 25 mL medium at 110–120 rpm in dark and at 25°C	<i>B. cereus</i> <i>B. cereus</i> (extract) <i>B. cereus</i> (root-bacteria co-culture supernatant collected on day 21)	0.2% 100 mg/L 5 mL (25% v/v)	0	28	Inhibit 50%	TT Volumetric TT	0.20 mg/g DW 1.40 mg/L 0.17 mg/g DW 1.15 mg/L	2.67 mg/g DW 10.4 mg/L 0.54 mg/g DW 5.35 mg/L	1.250 660 100 250 400 360	[1]	
	ATCC 15834	MS medium	0.3 g fresh hairy roots 25 mL	<i>B. cereus</i> (Bacterial cells) <i>B. cereus</i> (extract) Bacterial polysaccharide (BPS)	2.5% 1g/L 100 mg/L	0 18	7	Inhibit 50% Inhibit 50%	TT Volumetric TT TT	0.15 mg/g DW 1.82 mg/L 0.17mg/g DW 0.19 mg/g DW	2.78 mg/g DW 22.4mg/L 2.04 mg/g DW 1.59 mg/g DW	1.700 1.100 1.100 700 900	[2]	
	C58C1	1/2 B5	1.0 g fresh roots in 100 mL medium at 180 rpm in dark and at 25°C	<i>Trichoderma atroviride</i> D16 extract of mycelium (EM) Polysaccharide fraction (PSF)	300 mg/L 150 mg/L 180 mg/L	21	18	Increase	DT-I CT	0.039 mg/g DW 0.037 mg/g DW 0.052 mg/g DW 0.053 mg/g DW	1.338 mg/g DW 3.061 mg/g DW 1.216 mg/g DW 1.216 mg/g DW	3.400 8.200 2.200 6.500	[3]	
	ATCC 15834	MS medium	Hairy roots (0.3 g FW) into 40 mL medium at 120	Endophytic bacteria <i>Pseudomonas brassicacearum</i> subsp. <i>neoaurantiaca</i> (B1)	0.025 %	21	9	Increase	TT DT-I CT	Not published Not published Not published	Not published Not published Not published	270 1.820 1.030	[4]	

					rpm at 25°C							
					and in the							
					dark							
ATCC	MS	Hairy roots	<i>Streptomyces pactum</i> Act12	2%	21	14	No	T-I	Not published	Not published	458	[5]
15834	medium	(0.3 g FW) into					difference	T-IIA	Not published	Not published	1.077	
		40 mL						DT-I	Not published	Not published	742	
		medium at 120						CT	Not published	Not published	1.221	
		rpm at 25°C						TT	Not published	Not published	819	
		and in the		4%			Slightly	T-I	Not published	Not published	467	
		dark					inhibit	T-IIA	Not published	Not published	936	
							9.89%	DT-I	Not published	Not published	1.431	
								CT	Not published	Not published	3.263	
								TT	Not published	Not published	1.161	
C58C1	1/2 B5		<i>Chaetomium globosum</i> D38	Not published	Not	18	Not	CT	Not published	Not published	1.390	[6]
	medium				published		published	DT-I	Not published	Not published	700	
			<i>Chaetomium globosum</i> D38	90 mg/L		18		CT	Not published	Not published	1.880	
			Extract of Mycelium (EM)	60 mg/L		18		DT-I	Not published	Not published	2.000	

Table S2. Effect of methyl jasmonate and coronatine on diterpenoids production in *in vitro* *Salvia* hairy roots cultures.

white fluorescent light (40 $\mu\text{mol m}^{-2} \text{s}^{-1}$)													
ATCC 15834	1/2 MS medium	Hairy roots (1 g) into 100 mL at 120 rpm, at 23°C and in the dark	MJ	100 μM	21	7	No difference	Aethiopinone	0.38 mg/g DW	9.72 mg/g DW	2,400	[13]	
					28	Inhibit	Ferruginol	1.93 mg/L	56.13 mg/L	2,800			
							Aethiopinone	4.40 mg/L	73.29 mg/L	1,500			
							Ferruginol	Not published	55.48 mg/L	-			
			Corona-tine	0.1 μM	7	No difference	Aethiopinone	0.38 mg/g DW	2.57 mg/g DW	600			
					28	No difference	Aethiopinone	4.40 mg/L	103.32 mg/L	2,300			
							Ferruginol	Not published	103.01 mg/L	5,300			
							Carnosic acid	Not published	36.75 mg/L	1,700			
S. castanea Del	ATCC 15834	6,7-V medium	Hairy roots were cultured in medium	MJ	200 μM	18	7	Increase	T-IIA	Not published	1.8 mg/g DW	99	[14]
								CT	0.34 mg/g DW	0.51 mg/g DW	50		

Table S3. Effect of elicitors on diterpenoids production in *in vitro* *Salvia* cell cultures.

<i>Salvia</i> <i>sp.</i>	Transformed or non	Culture medium	Growth condition	Elicitors	Conc. of elicitor	Elicited on day	Time of elicitation (days)	Biomass growth vs CTRL	Diterpenes	Yield of control	Yield after elicitation	% increase over the control	References
<i>S. miltiorrhiza</i>													
	<i>A. tumefaciens</i>	MS medium without NH ₄ NO ₃	Fresh cell culture (ca. 0.4 g) into 20 mL medium in darkness at 25 °C	YE	4 g/L	7	15	Inhibit	TT (CT, T-IIA)	trace	12.23 mg/L	-	[15]
		B5 medium	3 g fresh weight in 100 mL at 140 rpm in darkness at 25°C for 16 days	YE	4 g/L	0	8	Inhibit	TT	Trace	20.1 mg/L	-	[16]
		6,7-V medium		YE	4 g/L	0	8	Inhibit	TT	Trace	22.2 mg/L	-	
		B5 medium	3.5 g cell into 50 mL medium at 140 rpm at dark and at 25°C	YE	0,1%	4	5	Inhibit	CT	0 mg/L	11.5 mg/L	-	[17]
	-	MS with 0.5 mg/L 2,4-D, 1 mg/L	Into 100 mL of at 27 ± 0.5 °C at 120 rpm in the dark.	SA	200 µM	18	7		CT	Data shown	not published	339	[18]
		KIN		Ag ⁺	60 µM	18	7		CT	Data shown	not published	1.707	
		MS medium with 2 mg/L 2,4-D and 2 mg/L 6-BA	0.3 g fresh cells in 25 ml medium at 110–120 rpm, at 25°C in the dark	SA	100 µM	18	7	Inhibit	CT	59.9 µg/g DW	390.0 µg/g DW	550	[19]
									T-IIA	57.6 µg/g DW	72.8 µg/g DW	30	
									Co	50 µM	18	7	Slightly inhibit
									CT	59.9 µg/g DW	263.7 µg/g DW	340	
									Cd	25 µM	18	7	Inhibit
									CT	59.9 µg/g DW	1,854.0 µg/g DW	3.000	

Table S4. Effect of yeast extract on diterpenoids production in *in vitro* *Salvia* hairy root cultures.

	MS	0.3 g fresh weight of roots into 40 mL medium	YE	100 mg/L	21	9	Inhibit	TT	146.4 µg/g DW	732.4 µg/g DW	400	[23]	
	MS	0.2 g fresh weight of roots in 25 mL medium	YE	25 mg/L	21	9	Inhibit	TT (CT, T-I, T-IIA)	0.2 mg/g DW	0.96 mg/g DW	380	[24]	
	medium	roots at 110–120 rpm						Volumetric	1.77 mg/L	7.62 mg/L	330		
	6,7-V	0.2 g fresh hairy roots into 50 mL at 110 rpm medium	YE	200 mg/L	18	6	Increase	TT	Not published	Not published	238	[25]	
	medium	in dark and at 25°C						CT	Not published	Not published	378		
<i>S. castanea</i> Dels	ATCC	6,7-V	Hairy roots were cultured in medium	YE	200 mg/L	18	7	Increase 73%	CT	Not published	2.84 mg/g DW	737	[14]
	15834	medium	into 50 mL at 110 rpm					T-IIA	Not published	2.52 mg/g DW	177		
		medium						DT-I	Not detected	1.95 mg/g DW	-		
		6,7-V	0.2 g fresh hairy roots into 50 mL at 110 rpm medium	YE	200 mg/L	18	6	Increase	DT-I	Not published	Not published	129	[25]
		medium	in dark and at 25°C					CT	Not published	Not published	800		
								T-I	Not published	Not published	3.614		
								T-IIA	Not published	Not published	232		

Table S5. Effect of Ag⁺ on diterpenoids production in *in vitro* *Salvia* hairy roots cultures.

<i>Salvia</i>	A.	Cultur	Growth condition	Elicitors	Conc.	Elicited	Time of	Biomass	Diterpenes	Yield	of	Yield after	% Increase over the	Reference
<i>a sp.</i>	Rhizogene	e			of	on day	elicitation	growth vs	stimulated	control		elicitation	control	s
	s	mediu			elicitor		n (days)	CTRL						
		m												
<i>S. miltiorrhiza</i>	ATCC	MS	0.3 g fresh root in 25 mL medium at 25°C	Ag ⁺	15 µM	18	12	Inhibit	TT	0.5 mg/g DW	2.3 mg/g DW	360	[26]	
	15834	mediu	mL medium at 110–120 rpm in dark and at 25°C					Volumetric	7.3 mg/L	25.3 mg/L	250			
		m						TT						
								Volumetric CT	1.76 mg/L	12.6 mg/L	500			
					Sucrose + Ag ⁺	15 µM	Suc. on day 9	Increase	Volumetric TT	Not published	Not published	54 compare to Ag ⁺		
							16 and Ag ⁺							
							on day 20							
					Medium renewal + Ag ⁺	15 µM	Suc. on day 9	Increase	Volumetric CT	Not published	Not published	50 compare to Ag ⁺		
							16 and Ag ⁺		Volumetric	Not published	Not published	120 compare to Ag ⁺		
							on day 20	T-IIA						
<i>S. miltiorrhiza</i>	MS	0.3 g fresh root in 25 mL medium at 25°C	Ag ⁺	30 µM	18	4	Inhibit	TT		Not published	Not published	20	[21]	
	mediu	mL medium at 110–120 rpm in dark and at 25°C												
		m												
<i>S. miltiorrhiza</i>	6,7-V	0.2 g fresh hairy roots in 50 mL medium at 110 rpm in the dark and at 25 °C	Ag ⁺	15 µM	18	6	No difference	DT-I	Not published	0.60 mg/g DW	156	[27]		
	mediu	into 50 mL at 110 rpm						T-I	Not published	0.82 mg/g DW	46			
		m						CT	Not published	0.34 mg/g DW	42			
								T-IIA	Not published	0.42 mg/g DW	340			
								TT	Not published	2.37 mg/g DW	120			
<i>S. miltiorrhiza</i>	6,7-V	0.2 g fresh hairy roots in dark and at 25°C	Ag ⁺	15 µM	18	6	No difference	DT-I	Not published	Not published	65	[25]		
	mediu	into 50 mL at 110 rpm						CT	Not published	Not published	64			
		m						T-I	Not published	Not published	85			

Table S6a. Effect of others chemical compounds on diterpenoids production in *in vitro* *Salvia* hairy roots cultures.

<i>Salvia</i> <i>sp.</i>	A. <i>Rhizogene</i> <i>s</i>	Culture medium	Growth condition	Elicitors	Conc. of elicitor	Elicited day	Time of elicitation	Biomass (days)	Diterpenes CTRL	Yield of control	Yield after elicitation	% over the control	References	
<i>S. multiorrhiza</i>		1/2 MS	Hairy roots into 50 mL	SA	100 µM	60		1.5	-	TT (T-I, T-IIA, CT, DT-I)	3.55 mg/g DW	5.95 mg/g DW	63	[9]
		medium	medium at 100 rpm in the dark and at 25°C											
ATCC	MS	0.2 g of fresh roots in 25 mL	Sorbitol	70 g/L	21		6		Slightly inc rease	TT	165.1 µg/g DW	723.6 µg/g DW	350	[23]
15834	medium	medium at 110–120 rpm in the dark and at 25°C		50 g/L					Volumetric	1.69 mg/L	8.18 mg/L	380		
ATCC	MS	0.2 g fresh weight of roots in 25 mL medium at 110–120 rpm	Sorbitol	50 mg/L	21		9		Increase	TT (CT, T-I, T-IIA)	0.2 mg/g DW	0.67 mg/g DW	235	[24]
15834	medium								Volumetric	1.96 mg/L	10.5 mg/L	436		
									TT					
						21	with	From days	No	TT (CT, T-I, T-IIA)	0.36 mg/g DW	3.22 mg/g DW	794	
						repeated		21 to day	difference					
						Sorbitol	and	60		Volumetric	3.01 mg/L	39.4 mg/L	1.200	
						nutrient			TT					
						feeding	every							
						5 days								
ATCC	MS	0.3 g fresh hairy roots into 50 mL at 110–120 rpm without NH ₄ NO ₃	Abscisica cid (ABA)	200 µM	18		6		Inhibit	T-I	0.7 mg/g DW	1.1 mg/g DW	110	[30]
15834	medium								CT	1.3 mg/g DW	1.6 mg/g DW	170		
									DT-I	3.2 mg/g DW	3.9 mg/g DW	60		
									T-IIA	1.5 mg/g DW	2.2 mg/g DW	90		

ATCC 15834	Not published	Not published	ABA	210 µM	20	7	Not published	T-I	Not published	Not published	440	[30]
	published						published	CT	Not published	Not published	850	
								DT-I	Not published	Not published	50	
								T-II A	Not published	Not published	80	
BCRC 15010	B5 liquid medium	(1.5 cm, ~5.4 mg dw) root in 70 mL medium at 100 rpm in the dark and at 25±2°C	(ABA) abscisica cid	1.0 mg/L	0	84	No difference	T-I T-IIA CT TT	0.216 mg/g DW 0.120 mg/g DW 0.374 mg/g DW 0.71 mg/g DW	0.452 mg/g DW 0.341 mg/g DW 1.038 mg/g DW 1.831 mg/g DW	109 200 200 150	[31]
			TDZ	1.0 mg/L			Increase	T-I T-IIA CT TT	0.216 mg/g DW 0.120 mg/g DW 0.374 mg/g DW 0.71 mg/g DW	0.393 mg/g DW 0.195 mg/g DW 1.310 mg/g DW 1,957 mg/g DW	100 50 250 170	

Table S6b. Effect of other elicitors on diterpenoids production in *in vitro* *Salvia* hairy root cultures.

<i>Salvia</i> <i>sp.</i>	A. <i>Rhizogenes</i>	Culture medium	Growth condition	Elicitors	Conc. of elicitor	Elicited on day	Time of elicitation n (days)	Biomass growth vs stimulated CTRL	Diterpenes	Yield of control	Yield after elicitation	% Increase over the control	Referenc
<i>S. miltiorrhiza</i>	ATCC 15834	MS medium	0.3 g fresh root in 25 mL medium at 110–120 rpm in dark and at 25°C	BABA	2 mM	18	4	Inhibit	TT (CT, T-I, T-IIA)	0.24 mg/g DW	1.09 mg/g DW	350	[32]
		MS medium	Hairy roots (0.3 g) in 50 mL medium at 110–120 rpm on an orbital shaker in darkness at 25°C	SNP	100 µM	18	6	No difference	T-I, CT, T-IIA	Not published	Not published	80	[30]
		MS medium	0.3 g fresh hairy roots into 50 mL at 110–120 rpm in darkness and 1 NH ₄ NO ₃ at 25°C	Polyethylene glycol (PEG)	2 % (w/v)	18	6	Inhibit	T-I CT DT-I T-IIA	0.7 mg/g DW 1.3 mg/g DW 3.2 mg/g DW 1.5 mg/g DW	0.9 mg/g DW 2.1 mg/g DW 3.9 mg/g DW 2.0 mg/g DW	70 100 60 70	[30]
ACCC 10060	6, 7-V	at 110–120 rpm at 25 ± 1 °C in darkness	Smoke–water (SW)	Smoke–water (SW)	SW 1:1000 (v/v)	18	3	Not published	T-I	Not published	Not published	230	[33]

Table S7. Effect of physical elicitors on diterpenoids production in *in vitro* *Salvia* hairy root cultures.

<i>Salvia</i>	A.	Culture	Growth condition	Elicitors	Conc. of elicitor	Elicited on day	Time of elicitation	Biomass growth vs	Diterpe nes	Yield of control elicitation	% over	Increase the	References
<i>sp.</i>	<i>Rhizogenes</i>	mediu m					n (days)	CTRL	stimula ted				control
<i>S. miltiorrhiza</i>	R1601	MS mediu m	Hairy roots in 50 mL medium at 120 rpm and at 25°C	UV-B	40 µW/cm²	18	40 min	TT CT	Not published Not published	0.38 mg/g DW 0.13 mg/g	80 240		[10]
LBA1334	1/2 B5	0.3 g fresh hairy roots in dish under LED light intensity of 110 µmol m⁻² s⁻¹ for 16 h per day	Light 9R (100% red) 110 µmol m⁻² s⁻¹ for 16 h per day 6R3IR (R:IR=100:26)	Light intensity of 110 µmol m⁻² s⁻¹ for 16 h per day 6R3IR (R:IR=100:26)	28	7	No difference	T-IIA	Not published Not published Not published	Not published Not published Not published	40 20 60		[34]

Table S8. Effect of combination of elicitors on diterpenoids production in *in vitro* *Salvia* hairy roots cultures.

							CT	Not published	Not published	290	
		YE+	2.5 mg/mL	18		5	Not published	DT-I	Not published	800	
		Ag+	+ 100 µM +				published	CT	Not published	Not published 660	
		MJ	200 µM,								
R1601	MS medium	Hairy roots in 50 mL medium at 120 rpm and at 25°C	UV-B + MJ	40 µW/cm ² after 100 µM MJ	18	UV (40 min) MJ (9 days)	Inhibit	CT T-I TT Volumetric TT	Not published Not published Not published Not published	0.97 mg/g DW 0.93 mg/g DW 2.26 mg/g DW 28.21 mg/L	890 510 570 390
						UV (40 min) MJ (7 days)	T-IIA	Not published	0.46 mg/g DW	240	

Table S9. Effect of combination of elicitors on diterpenoids production in *in vitro* *Salvia* cell cultures.

<i>Salvi</i> <i>a sp.</i>	Transforme d or non	Culture medium	Growth condition	Elicitors	Conc. of elicitor	Elicited on day	Time of elicitatio n (days)	Biomass growth vs CTRL	Diterpenes stimulated	Yield of control	Yield after elicitation	% Increase over the control	Referenc	
<i>S. miltorrhiza</i>														
	A. <i>tumefaciens</i>	B5	3 g fresh weight in 100 mL at 140 rpm in darkness at 25°C for 16 days	YE + light (L) YE + dark (D)	4 g/L + 4000 lux. 4 g/L	0	8	Data not shown	TT	No detectable	1.92 mg/L	-	[16]	
			3.5 g of fresh cell into 50 mL medium containing 20 g sucrose/L at 140 rpm in darkness and at 25°C	YE + SA (v/v) + SA	0.1% after add YE	3 and 24 h	5	Inhibit	CT	0 mg/L	18.9 mg/L	37	[17]	
	-	MS	Fresh cell culture (ca. 0.4 g) into 20 mL medium in darkness at 25 °C	YE + SA	0.12 mL + NH ₄ NO ₃	7	200 μM	15	Inhibit	TT (CT, T-IIA)	Hardly detectable	15.07 mg/L	-	[15]

References

- Wu, J.-Y.; Ng, J.; Shi, M.; Wu, S.-J. Enhanced secondary metabolite (tanshinone) production of *Salvia miltorrhiza* hairy roots in a novel root–bacteria coculture process. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 543–550.
- Zhao, J.L.; Zhou, L.G.; Wu, J.Y. Promotion of *Salvia miltorrhiza* hairy root growth and tanshinone production by polysaccharide–protein fractions of plant growth-promoting rhizobacterium *Bacillus cereus*. *Process Biochem.* **2010**, *45*, 1517–1522.

3. Ming, Q.; Han, T.; Li, W.; Zhang, Q.; Zhang, H.; Zheng, C.; Huang, F.; Rahman, K.; Qin, L. Tanshinone IIA and tanshinone I production by *Trichoderma atroviride* D16, an endophytic fungus in *Salvia miltiorrhiza*. *Phytomedicine* **2012**, *19*, 330–333.
4. Yan, Y.; Zhang, S.; Zhang, J.; Ma, P.; Duan, J.-L.; Liang, Z. Effect and mechanism of endophytic bacteria on growth and secondary metabolite synthesis in *Salvia miltiorrhiza* hairy roots. *Acta Physiol. Plant.* **2014**, *36*, 1095–1105.
5. Yan, Y.; Zhang, S.; Yang, D.; Zhang, J.; Liang, Z. Effects of *Streptomyces pactum* Act12 on *Salvia miltiorrhiza* hairy root growth and tanshinone synthesis and its mechanisms. *Appl. Biochem. Biotechnol.* **2014**, *173*, 883–893.
6. Zhai, X.; Luo, D.; Li, X.; Han, T.; Jia, M.; Kong, Z.; Ji, J.; Rahman, K.; Qin, L.; Zheng, C. Endophyte *Chaetomium globosum* D38 promotes bioactive constituents accumulation and root production in *Salvia miltiorrhiza*. *Front. Microbiol.* **2018**, *8*, 2694.
7. Wang, X.; Cui, G.; Huang, L.; Qiu, D. Effects of methyl jasmonat on accumulation and release of tanshinones in suspension cultures of *Salvia miltiorrhiza* hairy root. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica* **2007**, *32*, 300–302.
8. Liang, Z.-S.; Yang, D.-F.; Liang, X.; Zhang, Y.-J.; Liu, Y.; Liu, F.-H. Roles of reactive oxygen species in methyl jasmonate and nitric oxide-induced tanshinone production in *Salvia miltiorrhiza* hairy roots. *Plant cell reports* **2012**, *31*, 873–883.
9. Hao, X.; Shi, M.; Cui, L.; Xu, C.; Zhang, Y.; Kai, G. Effects of methyl jasmonate and salicylic acid on tanshinone production and biosynthetic gene expression in transgenic *Salvia miltiorrhiza* hairy roots. *Biotechnol Appl Biochem* **2015**, *62*, 24–31, doi:10.1002/bab.1236.
10. Wang, C.H.; Zheng, L.P.; Tian, H.; Wang, J.W. Synergistic effects of ultraviolet-B and methyl jasmonate on tanshinone biosynthesis in *Salvia miltiorrhiza* hairy roots. *Journal of photochemistry and photobiology. B, Biology* **2016**, *159*, 93–100, doi:10.1016/j.jphotobiol.2016.01.012.
11. Xing, B.; Yang, D.; Liu, L.; Han, R.; Sun, Y.; Liang, Z. Phenolic acid production is more effectively enhanced than tanshinone production by methyl jasmonate in *Salvia miltiorrhiza* hairy roots. *Plant Cell, Tissue and Organ Culture (PCTOC)* **2018**, *134*, 119–129.
12. Kuźma, Ł.; Bruchajzer, E.; Wysokińska, H. Methyl jasmonate effect on diterpenoid accumulation in *Salvia sclarea* hairy root culture in shake flasks and sprinkle bioreactor. *Enzyme Microb. Technol.* **2009**, *44*, 406–410.
13. Vaccaro, M.C.; Maria Evelina, A.; Malafronte, N.; De Tommasi, N.; Leone, A. Increasing the synthesis of bioactive abietane diterpenes in *Salvia sclarea* hairy roots by elicited transcriptional reprogramming. *Plant Cell Rep.* **2017**, *36*, 375–386.
14. Li, B.; Wang, B.; Li, H.; Peng, L.; Ru, M.; Liang, Z.; Yan, X.; Zhu, Y. Establishment of *Salvia castanea* Diels f. *tomentosa* Stib. hairy root cultures and the promotion of tanshinone accumulation and gene expression with Ag(+), methyl jasmonate, and yeast extract elicitation. *Protoplasma* **2016**, *253*, 87–100, doi:10.1007/s00709-015-0790-9.
15. Li, G.-J.; Wang, S.-C.; Xia, K.; Zhou, X. Effect of yeast elicitor and salicylic acid on the fluctuation of phytohormone contents in Ti-transformed *Salvia miltiorrhiza* cell cultures. *Plant Growth Regul.* **2003**, *39*, 27–32.

16. Chen, H.; Yuan, J.-P.; Chen, F.; Zhang, Y.-L.; Song, J.-Y. Tanshinone production in Ti-transformed *Salvia miltiorrhiza* cell suspension cultures. *J. Biotechnol.* **1997**, *58*, 147–156.
17. Chen, H.; Chen, F. Effects of methyl jasmonate and salicylic acid on cell growth and cryptotanshinone formation in Ti transformed *Salvia miltiorrhiza* cell suspension cultures. *Biotechnol. Lett.* **1999**, *21*, 803–807.
18. Yu, Y.; Wang, T.; Wu, Y.; Zhou, Y.; Jiang, Y.; Zhang, L. Effect of elicitors on the metabolites in the suspension cell culture of *Salvia miltiorrhiza* Bunge. *Physiol. Mol. Biol. Plants* **2019**, *25*, 229–242.
19. Zhao, J.-L.; Zhou, L.-G.; Wu, J.-Y. Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. *Applied Microbiology and Biotechnology* **2010**, *87*, 137–144.
20. Chen, H.; Chena, F.; Chiu, F.C.; Lo, C.M. The effect of yeast elicitor on the growth and secondary metabolism of hairy root cultures of *Salvia miltiorrhiza*. *Enzyme Microb Technol* **2001**, *28*, 100–105.
21. Ge, X.; Wu, J. Tanshinone production and isoprenoid pathways in *Salvia miltiorrhiza* hairy roots induced by Ag⁺ and yeast elicitor. *Plant science* **2005**, *168*, 487–491.
22. Yan, Q.; Hu, Z.; Tan, R.X.; Wu, J. Efficient production and recovery of diterpenoid tanshinones in *Salvia miltiorrhiza* hairy root cultures with in situ adsorption, elicitation and semi-continuous operation. *J. Biotechnol.* **2005**, *119*, 416–424.
23. Shi, M.; Kwok, K.W.; Wu, J.Y. Enhancement of tanshinone production in *Salvia miltiorrhiza* Bunge (red or Chinese sage) hairy-root culture by hyperosmotic stress and yeast elicitor. *Biotechnol Appl Biochem* **2007**, *46*, 191–196, doi:10.1042/BA20060147.
24. Wu, J.-Y.; Shi, M. Ultrahigh diterpenoid tanshinone production through repeated osmotic stress and elicitor stimulation in fed-batch culture of *Salvia miltiorrhiza* hairy roots. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 441–448.
25. Yang, D.; Fang, Y.; Xia, P.; Zhang, X.; Liang, Z. Diverse responses of tanshinone biosynthesis to biotic and abiotic elicitors in hairy root cultures of *Salvia miltiorrhiza* and *Salvia castanea* Diels f. *tomentosa*. *Gene* **2018**, *643*, 61–67.
26. Zhang, C.; Yan, Q.; Cheuk, W.-k.; Wu, J. Enhancement of tanshinone production in *Salvia miltiorrhiza* hairy root culture by Ag⁺ elicitation and nutrient feeding. *Planta Med.* **2004**, *70*, 147–151.
27. Xing, B.; Yang, D.; Guo, W.; Liang, Z.; Yan, X.; Zhu, Y.; Liu, Y. Ag⁺ as a more effective elicitor for production of tanshinones than phenolic acids in *Salvia miltiorrhiza* hairy roots. *Molecules* **2015**, *20*, 309–324.
28. Bian, L.; Zou, L.; Zhou, B.; Liu, W.; Zhou, J.; Wang, X. Effect of Lanthanum on accumulation of active constituent and key enzymes expression of *Salvia miltiorrhiza* hairy root. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica* **2016**, *41*, 4344–4349.
29. Han, M.; Guo, W.; Liang, Z.; Yang, D.; Yan, X.; Zhu, Y.; Liu, Y. Effects of cerous nitrate on growth and tanshinone production in *Salvia miltiorrhiza* hairy roots. *J. Rare Earths* **2015**, *33*, 1228–1235.

30. Yang, D.; Sheng, D.; Duan, Q.; Liang, X.; Liang, Z.; Liu, Y. PEG and ABA trigger the burst of reactive oxygen species to increase tanshinone production in *Salvia miltiorrhiza* hairy roots. *J. Plant Growth Regul.* **2012**, *31*, 579–587.
31. Gupta, S.K.; Liu, R.-B.; Liaw, S.-Y.; Chan, H.-S.; Tsay, H.-S. Enhanced tanshinone production in hairy roots of '*Salvia miltiorrhiza* Bunge' under the influence of plant growth regulators in liquid culture. *Bot Stud* **2011**, *52*, 435–443.
32. Ge, X.; Wu, J. Induction and potentiation of diterpenoid tanshinone accumulation in *Salvia miltiorrhiza* hairy roots by β -aminobutyric acid. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 183–188.
33. Zhou, J.; Ran, Z.; Xu, Z.; Liu, Q.; Huang, M.; Fang, L.; Guo, L. Effects of smoke-water and smoke-isolated karrikinolide on tanshinones production in *Salvia miltiorrhiza* hairy roots. *S. Afr. J. Bot.* **2018**, *119*, 265–270.
34. Chen, I.J.; Lee, M.S.; Lin, M.K.; Ko, C.Y.; Chang, W.T. Blue light decreases tanshinone IIA content in *Salvia miltiorrhiza* hairy roots via genes regulation. *Journal of photochemistry and photobiology. B, Biology* **2018**, *183*, 164–171, doi:10.1016/j.jphotobiol.2018.04.013.
35. Cheng, Q.; He, Y.; Li, G.; Liu, Y.; Gao, W.; Huang, L. Effects of combined elicitors on tanshinone metabolic profiling and SmCPS expression in *Salvia miltiorrhiza* hairy root cultures. *Molecules* **2013**, *18*, 7473–7485, doi:10.3390/molecules18077473.