

Supplementary File

Supplementary Table S1

Table S1. B5 solid medium composition.

Nutrient component	Concentration (mg/L)
Sucrose	20000
Agar	7000
KNO ₃	3000
Hydrolyzed milk protein	500
MgSO ₄ ·7H ₂ O	500
Inositol	200
NaH ₂ PO ₄ ·H ₂ O	150
CaCl ₂ ·2H ₂ O	150
(NH ₄) ₂ SO ₄	134
Na ₂ EDTA	37.3
FeSO ₄ ·7H ₂ O	27.8
Vitamin B ₁	20
MnSO ₄ ·H ₂ O	10
H ₃ PO ₃	3
Vitamin B ₆	3
ZnSO ₄ ·7H ₂ O	2
Niacin	2
Glycine	2
α-Naphthalene Acetic Acid	2
KI	0.75
Na ₂ MoO ₄ ·2H ₂ O	0.25
CuSO ₄ ·5H ₂ O	0.025
CoCl ₂ ·6H ₂ O	0.025

Supplementary Table S2

Table S2. Dry weight (DW), fresh weight (FW), relative electrolytic leakage and paclitaxel production under PEG, CD, or SA treatment in *Taxus cuspidata* suspension cells.

Treatment	DW (g/L)	FW (g/L)	Relative electrolytic leakage (%)	Paclitaxel (mg/L)
1.5% PEG	13.20±0.31	142.19±0.42	29.65±1.43	1.62±0.11
3% PEG	13.81±0.30	150.00±0.59	31.63±1.95	2.55±0.15
4.5% PEG	13.49±0.58	140.38±0.60	49.01±1.76	1.85±0.14
6% PEG	8.09±0.88	119.95±0.63	62.52±1.47	1.63±0.12
25 mmol/L CD	11.99±1.24	147.41±7.56	25.66±1.13	1.73±0.14
50 mmol/L CD	14.98±1.21	171.55±8.25	24.29±1.65	2.40±0.15
75 mmol/L CD	14.07±0.60	157.03±8.18	27.28±2.57	2.30±0.12
100 mmol/L CD	9.62±0.89	142.40±6.57	33.69±2.21	1.95±0.12
5 mg/L SA	11.69±0.31	129.38±7.19	32.52±4.58	2.10±0.10
10 mg/L SA	11.41±0.28	119.01±8.20	43.58±2.74	2.69±0.10
15 mg/L SA	11.11±0.31	115.22±8.38	61.04±5.98	3.20±0.12
20 mg/L SA	10.80±0.29	100.78±5.60	72.34±3.49	3.56±0.15

Supplementary Table S3

Table S3. Response surface test design and results.

	A-PEG (%)	B-CD (mmol/L)	C-SA (mg/L)	Paclitaxel (mg/L)
1	-1 (2)	1 (65)	0 (20)	2.87
2	0 (3)	0 (50)	0	6.92
3	0	-1 (35)	1 (30)	4.18
4	0	1	-1 (10)	5.14
5	0	0	0	7.52
6	1 (4)	0	-1	7.81
7	-1	-1	0	4.83
8	0	0	0	8.11
9	1	0	1	3.99
10	0	0	0	7.67
11	-1	0	-1	4.04
12	0	-1	-1	6.21
13	1	1	0	5.02
14	0	0	0	6.94
15	1	-1	0	6.03
16	0	1	1	3.35
17	-1	0	1	4.00

Supplementary Table S4

Table S4. The analysis of variance (ANOVA) for response surface polynomial model.

Source	Sum of squares	df	Mean square	F value	p-value
Model	45.30	9	5.03	29.54	<0.0001***
A-PEG	6.32	1	6.32	37.09	0.0005***
B-CD	2.96	1	2.96	17.40	0.0042***
C-SA	7.37	1	7.37	43.27	0.0003***
AB	0.23	1	0.23	1.32	0.2876
AC	3.57	1	3.57	20.97	0.0025***
BC	0.014	1	0.014	0.085	0.7797
A ²	6.60	1	6.60	38.75	0.0004***
B ²	9.38	1	9.38	55.03	0.0001***
C ²	6.26	1	6.26	36.77	0.0005***
Residual	1.19	7	0.17		
Lack of fit	0.16	3	0.055	0.21	0.8827
Pure error	1.03	4	0.26		
Cor total	46.49	16			

R²=0.9743 AdjR²=0.9414 C.V.=7.42% AQ=15.166

*Significant at P < 0.05, ** Significant at P < 0.01, *** Significant at P < 0.001

Supplementary Figure S1

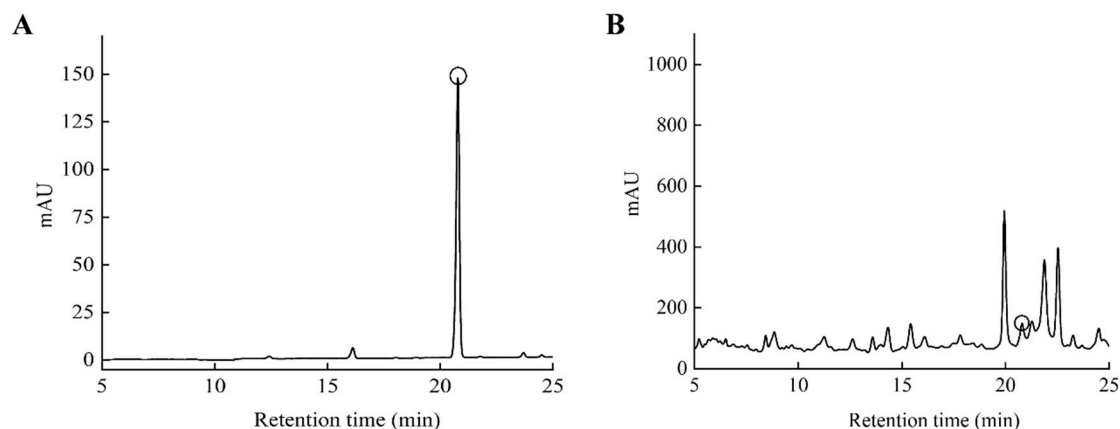


Figure S1. HPLC chromatograms of paclitaxel standard (A) and paclitaxel production by *T. cuspidata* suspension cells under elicitor treatments (B).

Supplementary Table S5

Table S5. Primers for quantitative real-time PCR analysis of genes involved in paclitaxel synthesis and associated with elicitors treatment.

Gene name	Sequence	Product sizes (bp)
TBC41	F: CAAGAAGAAAGAGTCAGCAAATGG R: GGAACGACATGACATTATGAATAGC	91
DXS:	F: GGGTTTTTCGTGTTTATCGGC	156
TRINITY_DN2395_c0_g1	R: CGTAAGCTTGTCCAGCAGTCAT	
DXR:	F: AACTAAGTGGCACTTTC	162
TRINITY_DN2533_c0_g1	R: AAGGAGCTCTGCGACGTA	
IspF:	F: ATGGCGGGATGCTAAAACG	207
TRINITY_DN497_c0_g1	R: CTCAATGTGGAGCAAACAAAGA	
IspG:	F: TCTCAACTGGTGCCACGA	212
TRINITY_DN2343_c0_g1	R: GGGGAAGTTCAGGGCGTTT	
IspH:	F: GGCATAGTAGATGGGGT	193
TRINITY_DN1245_c0_g1	R: TGATGTGTGCGTGTGAGA	
GGPPS:	F: TATCCTTTCCTTCGCCTTC	147
TRINITY_DN33697_c0_g4	R: AGAGAATGGCTTACACGGCA	
TASY:	F: AGCCGATGATGTCCCGAT	143
TRINITY_DN9815_c0_g1	R: AGTCCTTAATCTCCTCGTT	
DBAT:	F: GATTCCATCGGGGTATTCTTG	143
TRINITY_DN1144_c1_g3	R: GGCGATTGGGATTGATGAAG	
BAPT:	F: ATACTCCTCGCCTTGTTTCACC	274
TRINITY_DN1024_c0_g1	R: GGCTCAGAAGGAAAGAAAATGG	
DBTNBT:	F: GAAATGTTGTCAGGGGCA	185
TRINITY_DN109516_c0_g1	R: AGCGGAATCAGGATGGAG	

Supplementary Table S6**Table S6.** Statistics of RNA-Seq reads.

Sample name	Read Sum	Base Sum	GC (%)	N (%)	Q20 (%)	Cycle Q20 (%)	Q30 (%)
C1	23523922	7058076600	45.21	0.00	97.95	100.00	93.89
C2	25446602	7633980600	44.65	0.00	98.07	100.00	94.14
C3	24828539	7448561700	45.25	0.00	97.86	100.00	93.73
T1	23133225	6939967500	44.74	0.00	98.13	100.00	94.28
T2	27767789	8330336700	44.37	0.00	97.73	100.00	93.36
T3	24226452	7267935600	44.59	0.00	97.87	100.00	93.72