Supplementary information

Materials and methods

Culture conditions and chemical treatments

G. lucidum was cultured in seed culture as previously described (Cui et al., 2017), then inoculated (10%, volume fraction) into fermentation culture at 28 ℃ for 7 d with shaking at 180 r/min. The fermentation culture containing the following components were prepared: malt extract (20 g/L), yeast extract (18 g/L), KH_2PO_4 (3 g/L), $MgSO_4$ (1.5 g/L), and vitamin B_1 (0.05 g/L) at an initial pH of 5.5.

All chemicals were obtained from Sigma (St Louis, MO, USA) and Solarbio (Beijing, China). ABA was added to the fermentation cultures on Day 4 of culture at concentrations of 0, 100, 200, 300, 400, and 500 μmol/L. After ABA treatment for 24 h, $CaCl_2$ (10 mmol/L), ethylene glycol bis (2-aminoethyl), tetraacetic acid (EGTA, 10 mmol/L), and $LaCl_3$ (10 mM) were added on Day 5, respectively, and incubated for 24 h for Ca^{2+} signaling analysis. Mycelium was collected on Day 6 of culture.

Measurement of total GT content

The mycelium was collected and mixed with 95% ethanol at a ratio of 1∶50 (volume fraction) and extracted twice for 20 min at 50 ℃ under ultrasound (400 W). The vanillin-glacial acetic acid-perchloric acid method was used for GT content determination. Successively, 0.1 mL of extracts was evaporated solvent with heating at 60 ℃, and then 0.2 mL of 5% vanillin-glacial acetic acid solution (mass fraction) and 0.5 mL of perchloric acid were added and incubated at 60℃ for 20 min, then cooled to the ambient temperature. The GT content was measured at 550 nm after 5 mL of glacial acetic acid was added.

Real-time quantitative polymerase chain reaction analysis

Total RNA preparation and RT-PCR were carried out as previously reported (Gu et al., 2017). The key genes of GT biosynthesis used were as follows: 3-hydroxy-3-methylglutaryl-CoA reductase *(hmgr*, accession number EU263989), squalene synthase (*sqs*, accession number DQ494674), and lanosterol synthase (*ls*, accession number FJ195872). The key genes involved in Ca^{2+} signaling included calcium-channel protein *cch1*, calcium transporting adenosine triphosphatase (ATPase), calcium-dependent mitochondrial carrier protein (CDMCP), and vacuolar calcium ion transporter, calcium/calmodulin-dependent protein kinase *cmkB* (selected via transcriptome sequencing; Table S1, Fig. S4). Relative abundance was determined using *gpd* and *β-Tub* as internal standards. The related primers are listed in Table S2.

Determination of cytosolic Ca^{2+} concentration

The sample was crushed and homogenized, and 1.0g of sample was added in a 40mL sampling bottle, fixed volume to 10mL with deionized water, and then extracted by ultrasound (400 W) for 30 min. The extracted solution was passed through a 0.45-μm filter membrane and prepared to be tested, $CaCl_2$ was measured as a standard.

The analysis of Ca^{2+} concentration was performed on an ion chromatograph (ECO-C, Metromhm, Switzerland). The sample was separated on a Metrosep C4-150 column (250 mm×4.6 mm). The mobile phase consisted of HNO_3 (1.0 mmol/L and dipicolinic acid (0.7 mmol/L), and the detected volume was 20 μL with an elution rate of 1.5 mL/min at a column temperature of 25 ℃. A conductance detector was used for measurement.

The Ca^{2+} concentration was expressed as follows:

Ca^{2+} concentration (μg/g dry weight)= $\frac{\text{detected concentration (μg/mL)}\times\text{fixed volume (mL)}}{\text{sample fresh weight (g)}}$/ratio of dry weight to fresh weight of mycelium (1).

Determination of antioxidant properties

A total of nine antioxidant indexes were determined, including the contents of H_2O_2, malondialdehyde (MDA), and glutathione (GSH), superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-P_X) activity, catalase (CAT) activity, peroxidase (POD) activity, and ascorbate peroxidase (APX) activity. The ability to resist $O_2^{\cdot-}$ was also measured. All these determined methods followed the manufacturers' instructions in the detection kits purchased from Nanjing Jiancheng Bioengineering Institute.

Statistical analysis

All experiments were carried out in at least triplicate, and the results were expressed as the mean±standard deviation (SD), n=3. Statistical analysis was performed using IBM SPSS statistics 20 and Duncan's multiple-range test (P<0.05).

References

Cui ML, Yang HY, He GQ, 2017. Apoptosis induction of colorectal cancer cells HTL-9 in vitro by the transformed products of soybean isoflavones by *Ganoderma lucidum*. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 18(12):1101-1112. https://doi.org/10.1631/jzus.B1700189

Gu L, Zhong X, Lian DH, et al., 2017. Triterpenoid biosynthesis and the transcriptional response elicited by nitric oxide in submerged fermenting *Ganoderma lucidum*. *Process Biochem*, 60:19-26. https://doi.org/10.1016/j.procbio.2017.05.029

Supplementary Tables

Table S1 GO annotation of Ca^{2+} signaling-related transcripts after ABA treatment (P<0.05)

ID	log_2(fold change)	*P*	Length	Swissprot	Non-Redundant protein sequence
TRINITY_DN1841_c0_g1	3.107	<0.001	5214	sp|Q9UTN1|OAC1_SCHPO Mitochondrial oxaloacetate transport protein OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) OX=284812 GN=oac1 PE=3 SV=1	VWO99184.1 **Calcium dependent mitochon-drial carrier protein** [*Ganoderma boninense*]
TRINITY_DN379_c0_g1	2.066	<0.001	9683	sp|P13586|ATC1_YEAST Calcium-transporting ATPase 1 OS=Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=PMR1 PE=1 SV=1	AFX00736.1 **calcium transporting ATPase** [*Ganoderma lucidum*]
TRINITY_DN1158_c0_g1	1.992	<0.001	2770	sp|Q9Y899|KCC1B_EMEND **Calcium/calmodulin-dependent protein kinase cmkB** OS=Emericella nidulans OX=162425 GN=cmkB PE=1 SV=1	PIL28908.1 transporter [*Ganoderma sinense* ZZ0214-1]
TRINITY_DN11101_c0_g1	3.525	<0.001	6710	sp|O14234|CCH1_SCHPO **Calcium-channel protein cch1** OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) OX=284812 GN=cch1 PE=3 SV=1	PIL32546.1 transporter [*Ganoderma sinense* ZZ0214-1]
TRINITY_DN13_c0_g1	1.665	<0.001	6726	sp|O59768|VCX1_SCHPO **Vacuolar calcium ion transporter** OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) OX=284812 GN=vcx1 PE=3 SV=1	PIL24302.1 hypothetical protein GSI_14055 [*Ganoderma sinense* ZZ0214-1]
TRINITY_DN1869_c0_g1	–2.187	<0.001	1789	sp|O59731|YHXB_SCHPO Uncharacterized J domain-	VWP00319.1 **Calcium/prot**

				containing protein C3E7.11c OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) OX=284812 GN=SPBC3E7.11c PE=3 SV=1	**on exchanger** [*Ganoderma boninense*]
TRINITY_DN1673_c0_g1	–2.202	<0.001	3842	sp|P42839|VNX1_YEAST Low affinity vacuolar monovalent cation/H(+) antiporter OS= Saccharomyces cerevisiae (strain ATCC 204508 / S288c) OX=559292 GN=VNX1 PE=1 SV=1	AVR29896.1 **putative calcium-hydrogen exchanger 4** [*Ganoderma lucidum*]
TRINITY_DN1040_c0_g1	–3.806	<0.001	5241	sp|P40977|PLC1_SCHPO 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase 1 OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) OX=284812 GN=plc1 PE=1 SV=1	AVM41526.1 **phospholipase C** [*Ganoderma lucidum*]
TRINITY_DN5301_c0_g1	–1.499	0.037	1845	sp|Q10063|EHS1_SCHPO **Calcium influx-promoting protein *ehs1*** OS=Schizosaccharomyces pombe (strain 972 / ATCC 24843) OX=284812 GN=ehs1 PE=3 SV=1	AVM41529.1 Mid [*Ganoderma lucidum*]

GO: Gene Ontology; ABA: abscisic acid; ID: identity; ATPase: adenosine triphosphatase.

Table S2 Primer sequences used for reverse transcription-polymerase chain reaction analysis

Gene ID	Primer name	Primer sequence (5′ to 3′)
gpd	Real-gpd-F	GATGAAGGACTGGCGTGGT
	Real-gpd-R	CCGTTGAGGCTGGGAATGAC
β-Tub	Real-β-Tub-F	GCGCTCTACGACATTTGCTT
	Real-β-Tub-R	ACGATGGAGACGAGGTGGTT
sqs	Real-sqs-F	ACAGTTGTCAGCGAAGAGC
	Real-sqs-R	CGTAGTGGCAGTAGAGGTTG
hmgr	Real-hmgr-F	GTCATCCTCCTATGCCAAAC
	Real-hmgr-R	GGGCGTAGTCGTAGTCCTTC
ls	Real-ls-F	CTTCCGCAAGCACTACCCG
	Real-ls-R	AGCAGATGCCCCACGAGCC
calcium-channel protein *cch1*	Real-cch1-F	CCCTCCTCGTTCTCCTCCATCATAG
	Real-cch1-R	CATCGTCCTCAACTTCTGGCTCATC
calcium-dependent mitochondrial carrier protein	Real-CDMCP-F	CTCCTCCTGTCCCACTCTCTCAAC
	Real-CDMCP-R	GCTTGCTGTCCAGTCTGTCTAACC
calcium transporting ATPase	Real-calcium transporting ATPase-F	ATTCGTCCTGTCCGCATTCGTAAC
	Real-calcium transporting ATPase-R	TCGTCCCAATTCTCGCAAACCTG
Vacuolar calcium ion transporter	Real-vacuolar calcium ion transporter-F	GAAGTCGGCGAAGAACAGAGTGAG
	Real-vacuolar calcium ion transporter-R	TGGATGGCGATGAAGAACAAGATGG
calcium/calmodulin-dependent protein kinase *cmkB*	Real-cmkB-F	CCCCGGAAGCATTGGTAAGT
	Real-cmkB-R	TGGGGCTCCCTGATTCCATA

ID: identity; F: forward; R: reverse.

Supplementary figures

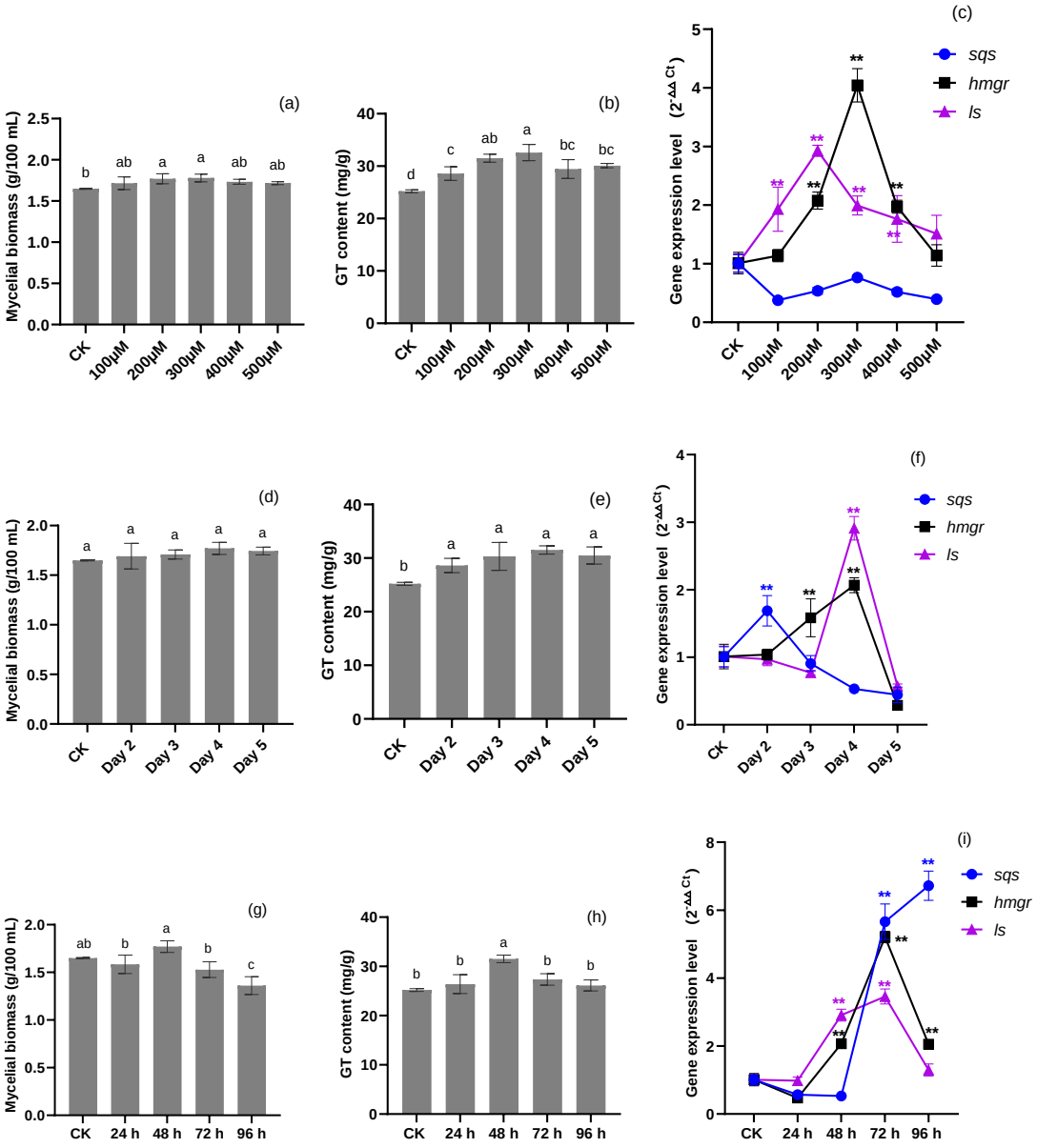


Fig. S1 Effects of different ABA concentrations (0, 100, 200, 300, 400, and 500 μmol/L) (a-c), different ABA addition time points (Day 2, 3, 4, and 5 of cultivation) (d-f), and ABA treatment durations (24, 48, 72, and 96 h) (g-i) on the biomass, GT accumulation and gene expression level of *G. lucidum*. Data were expressed as the mean±standard deviation (SD), *n*=3. Different lowercase letters indicate significant differences among various treatments (*P*<0.05). ***P*<0.01. ABA: abscisic acid; CK: contrast check; GT: ganoderic triterpenoids; *hmgr*: 3-hydroxy-3-methylglutaryl-CoA reductase; *ls*: lanosterol synthase; *sqs*: squalene synthase.

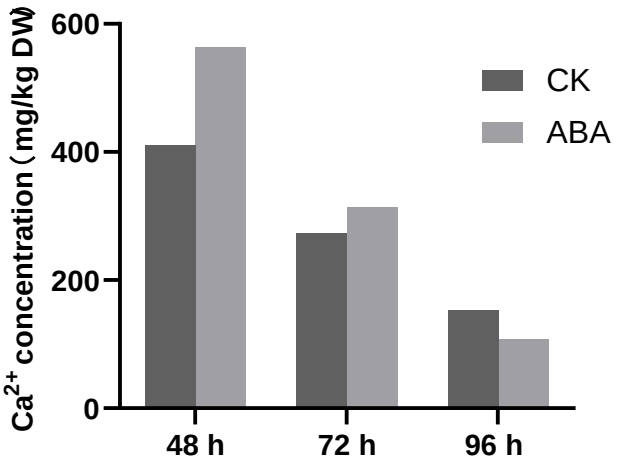
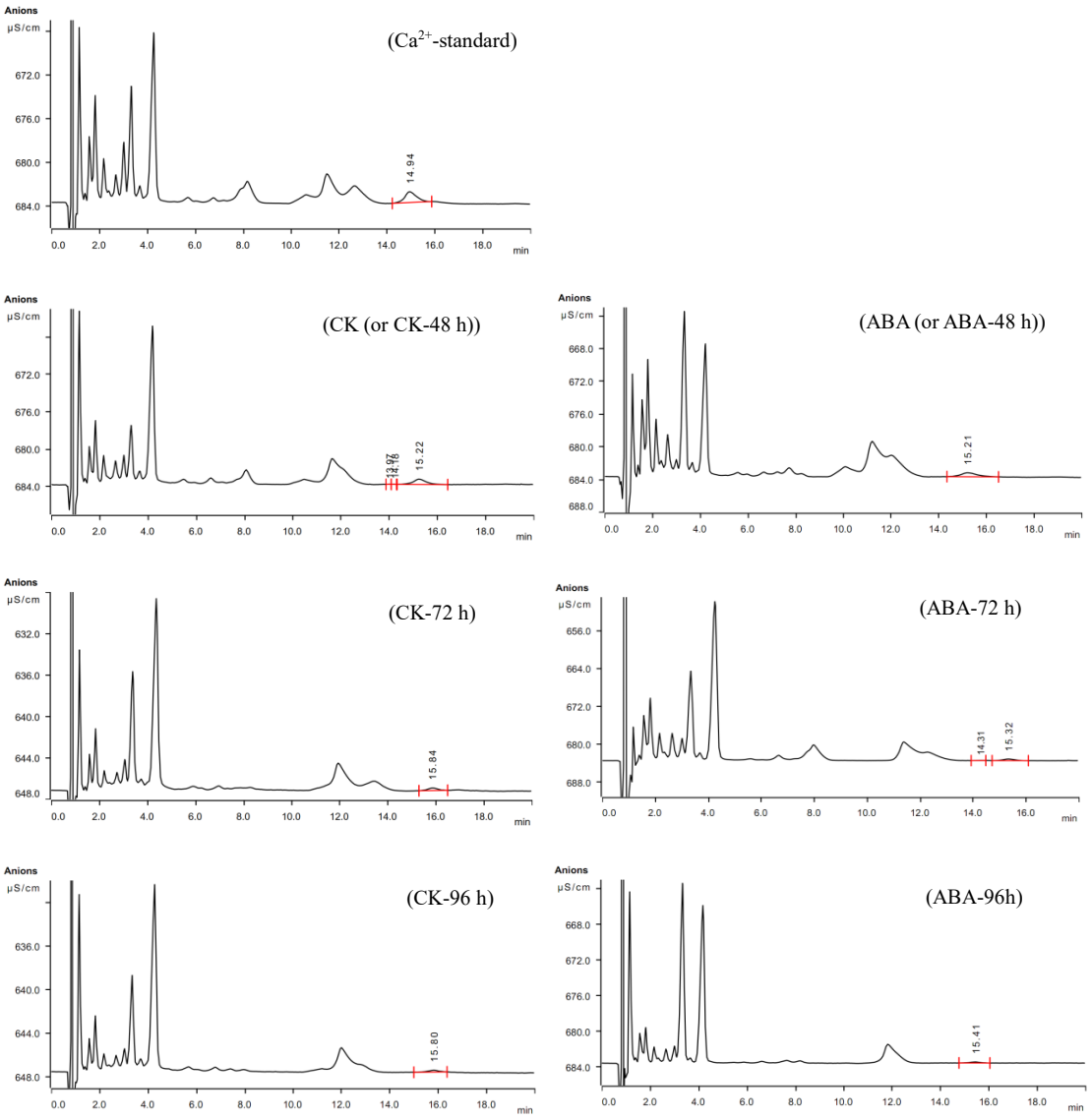


Fig. S2 Changes of cytosolic Ca^{2+} concentration determinating by ion chromatograph with ABA treatment for 48 to 96 h of *G. lucidum.* ABA: abscisic acid; CK: contrast check; DW: dry weight.



Sample	Retention time (min)	Ca^{2+} concentration (mg/kg wet weight)	Ratio of dry weight to wet weight of mycelium	Ca^{2+} concentration (mg/kg dry weight)
Ca^{2+}-standard	14.767	7.682		
CK	15.218	39.742	0.097	409.711
CK-72 h	15.838	13.968	0.051	273.160
CK-96 h	15.798	10.197	0.067	153.051
ABA	15.208	46.706	0.083	563.485
ABA-72 h	15.315	19.916	0.063	313.677
ABA-96 h	15.412	7.015	0.065	108.061

Fig. S3 Determination of cytosolic Ca^{2+} concentration by ion chromatograph with ABA treatment for 48 to 96 h of *G. lucidum*. ABA: abscisic acid; CK: contrast check.

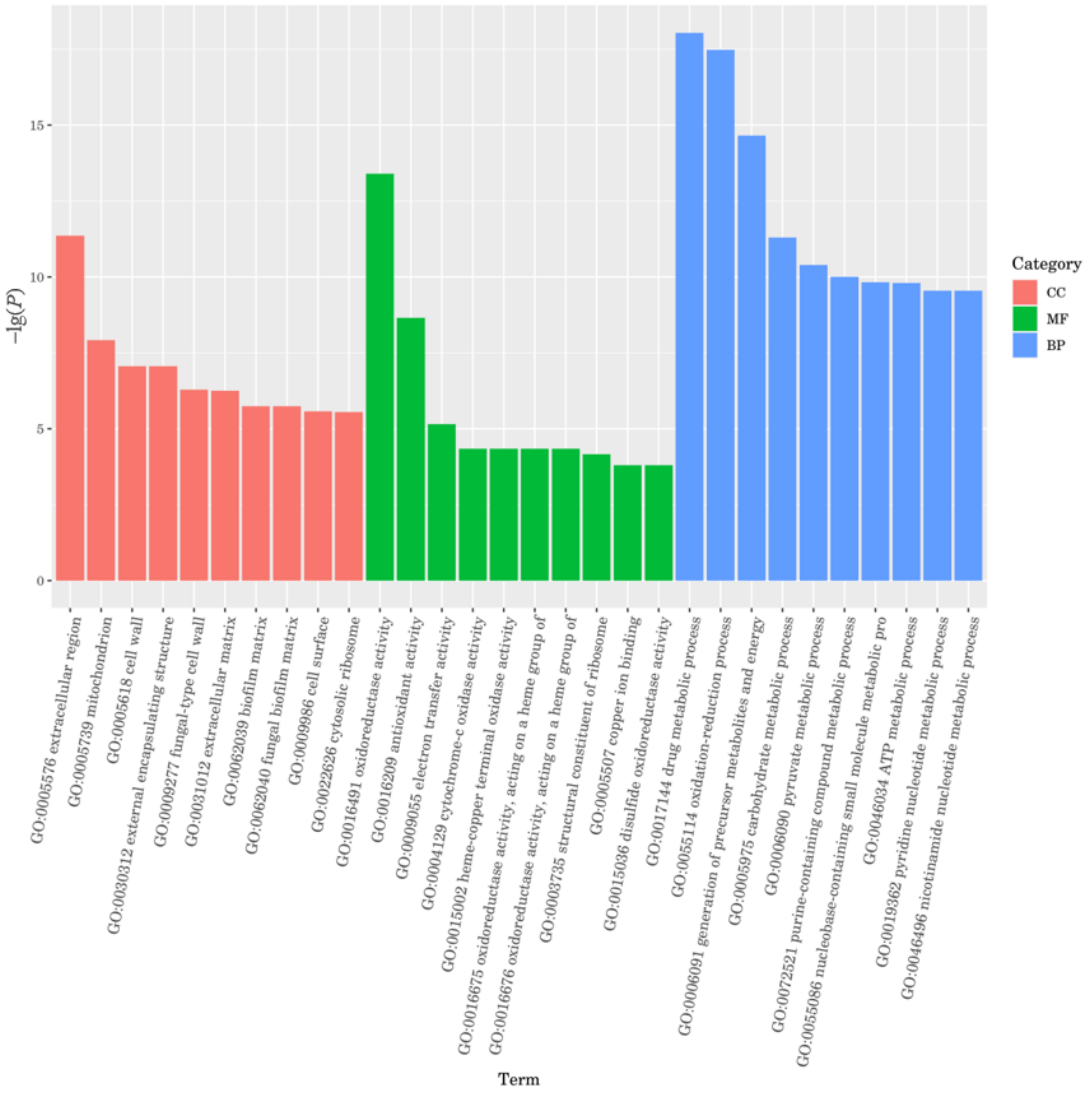
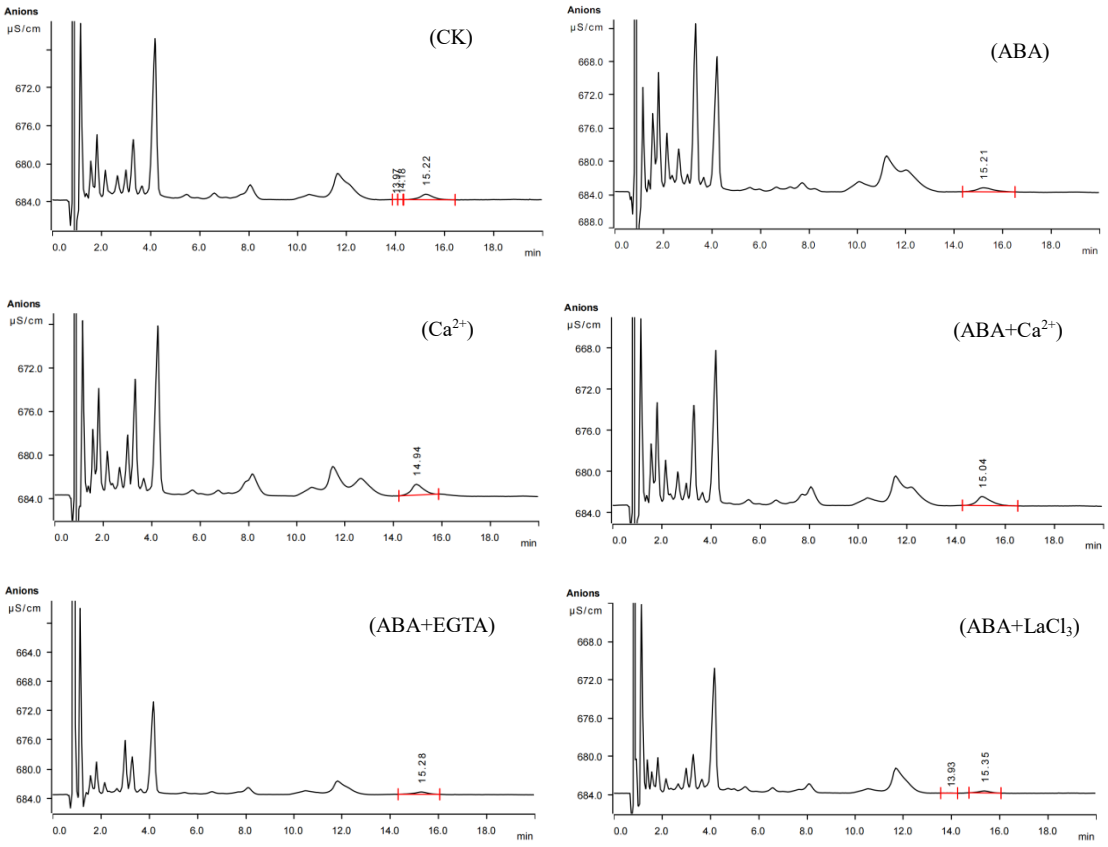


Fig. S4 GO categories of differentially expressed unigenes identified after ABA treatment relative to control. Gene classification based on GO annotation, −lg(*P*) was plotted on the Y-axis and the names of clusters were plotted on X-axis. GO: Gene Ontology; CC: cell component; MF: molecular function; BP: biological process.



Sample	Retention time (min)	Ca^{2+} concentration (mg/kg wet weight)	Ratio of dry weight to wet weight of mycelium	Ca^{2+} concentration (mg/kg dry weight)
CK	15.218	39.742	0.097	409.711
ABA	15.208	46.706	0.083	563.485
Ca^{2+}	14.935	66.768	0.063	1062.946
ABA+Ca^{2+}	15.042	69.025	0.056	1227.111
ABA+EGTA	15.283	24.128	0.048	505.998
ABA+$LaCl_3$	15.352	13.074	0.145	90.428

Fig. S5 Determination of cytosolic Ca^{2+} concentration by ion chromatograph with different treatments mediated by ABA. ABA: abscisic acid; CK: contrast check; EGTA: ethylene glycol tetraacetic acid.