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ORIGINAL ARTICLE

Enhancement of stilbene compounds and anti-inflammatory activity of methyl jasmonate and cyclodextrin elicited peanut hairy root culture

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Abstract

A highly stable and productive hairy root culture from peanut cultivar Tainan9 (T9-K599) was established using *Agrobacterium rhizogenes* strain K599 (NCPPB 2659)-mediated transformation. Valuable phenolic compounds with antioxidant activity and stilbene compounds were produced and secreted into the culture medium after elicitation with 100 µM methyl jasmonate (MeJA) and 6.87 mM cyclodextrin (CD). The antioxidant activity of the culture medium was increased to the highest Trolox equivalent antioxidant capacity (TEAC) value (28.30 ± 2.70 mM Trolox/g DW) in the group treated with CD. The group co-treated with MeJA and CD exhibited the highest phenolic content, with a gallic acid equivalent (GAE) value of 10.80 ± 1.00 µg gallic acid/g DW. The *CuZn-SOD* (CuZn superoxide dismutase) and *APX* (ascorbate peroxidase) antioxidant enzyme gene were up-regulated in the treatment with CD alone while the CuZn-*SOD, GPX* (glutathione peroxidase) and *APX* gene expression were down-regulated in the co-treatment with MeJA plus CD. The stilbene compounds resveratrol, trans-arachidin-1 and trans-arachidin-3 were detected by analysing the culture medium treated with CD alone and after co-treatment with MeJA and CD via HPLC. The LC-MS/MS results confirmed the presence of resveratrol, trans-arachidin-1, trans-arachidin-3, 4-Isopentadienyl-3,5,3′,4′-tetrahydroxystilbene (IPP), trans-3′-Isopentadienyl-3,5,4′-trihydroxystilbene (IPD) and arahypin-7. The results indicate that elicited peanut hairy roots can produce beneficial stilbene compounds that have antioxidant properties and anti-inflammatory activity. This peanut hairy root system could be applied as an experimental model to enhance the production of stilbene and other polyphenolic bioactive compounds.

Keywords Peanut hairy root · Stilbene · Resveratrol · Arachidin · Antioxidant activity · Anti-inflammatory

Introduction

Reactive oxygen species (ROS) has been reported to be associated with several human diseases such as diabetes, atherosclerosis, cancer, neurodegenerative disease, and aging (Ray et al. 2012). ROS molecules such as superoxide anion ($O2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\cdot}$), are highly unstable and induce the oxidation of biomolecules such as DNA, proteins and lipids which can induce a variety of chronic and degenerative diseases. The well-developed defence mechanisms to counteract the ROS and oxidative stress in plant are enzymatic antioxidant and non-enzymatic antioxidant process. The efficient enzymatic antioxidant system plays a crucial role in protecting cells from oxidative damage. These enzymes include the superoxide dismutase (*SOD*), catalase (*CAT*), glutathione peroxidase (*GPX*) and ascorbate peroxidase (*APX*). The

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non-enzymatic antioxidant belongs to antioxidant compounds, such as glutathione, vitamin E, vitamin C, flavonoids and polyphenols (Birben et al. 2012).

Polyphenol stilbene compounds, such as resveratrol, arachidin, piceatannol, and pterostilbene, are natural phenolic compounds produced by several plant families. The most prominent plant families to produce stilbenes are Vitaceae, Fabaceae, Gnetaceae and Dipterocarpaceae (Rivière et al. 2012). Peanut (*Arachis hypogaea*) belongs to the Fabaceae family and is considered to be one of the plant species that is capable of stilbene compound production (Hasan et al. 2013). The major stilbenoids reported in peanut are resveratrol and the prenylated products arachidin-1 and arachidin-3. These compounds are considered to be plant phytoalexins, which are part of the plant defence mechanism to protect against biotic and abiotic stress. Peanuts treated with a combination of ultrasound followed by UV light exhibited an increased resveratrol content compared to untreated peanut control (Potrebko and Resurreccion 2009). The resveratrol content and resveratrol synthase gene expression have been investigated in field-grown peanuts and are correlated with paraquat, wounding, H_2O_2, salicylic acid, jasmonic acid and ethephon treatment (Chung et al. 2003). Additionally, biotic stresses, such as the fungi *Aspergillus flavus, A. niger, A. caelatus*, and *A. nomius* as well as the bacteria *Bacillus subtilis* and *Rhizobium leguminosarum*, selectively elicit the production of resveratrol, arachidin-1, and arachidin-3 in peanut seeds and embryos (Sobolev 2013).

The stilbene compounds have received much attention due to their potentially beneficial health properties. Resveratrol has antioxidant, anti-inflammatory, and anticarcinogenic properties. Recent reports have indicated that resveratrol directly inhibits cyclic adenosine monophosphate-specific phosphodiesterases and activates 5′-adenosine monophosphate-activated kinase, thus increasing the potential for resveratrol application in cardiovascular and metabolic health (Xu and Si 2012). There is growing data shows that resveratrol acts as a potent colorectal cancer chemopreventive agent. Consumption of resveratrol reduces tumour cell proliferation. The results of a previous study suggested that daily consumed doses of resveratrol at 0.5 or 1.0 g can reduce tumour cell proliferation in colorectal cancer patients by 5% (Patel et al. 2010). In addition to resveratrol, the stilbenoid arachidin-1 demonstrates anticancer activity by inducing caspase-independent programmed cell death in human leukemia HL-60 cells with a four-fold lower EC50 than resveratrol (Huang et al. 2010). Molecular modelling studies have indicated that the prenylated products of arachidin-1 and arachidin-3 improve binding affinity to cannabinoid receptors (CBR). This observation demonstrates that CBR antagonists produce a variety of therapeutic effects, such as anti-obesity, anti-carcinogenic, and anti-inflammatory activities (Brents et al. 2012).

The hairy root culture system is a powerful biotechnology that can be applied for plant secondary metabolite production. Hairy root cultures transformed by *Agrobacterium rhizogenes* grow rapidly on phytohormone-free medium. Hairy roots are useful to produce biologically active molecules because large amounts can be produced with high cost effectiveness (Mallikarjuna et al. 2016). Resveratrol production from hairy root cultures has been extensively studied in several plants, such as *Scutellaria baicalensis* (Lee et al. 2013) and *Vitis rotundifolia michx* (Nopo-Olazabal et al. 2013). Hairy root cultures of peanut have been established to produce resveratrol and its derivatives. Hairy root cultures, along with the sodium acetate elicitation approach, lead to a 60-fold induction and secretion of resveratrol into the medium of peanut hairy root cultures (Medina-Bolivar et al. 2007). The most promising strategy to enhance the production of resveratrol, piceatannol, arachidin-1, and arachidin-3 is co-treatment with methyl jasmonate and cyclodextrin, which promotes a high resveratrol yield in peanut hairy root cultures (Yang et al. 2015).

In this study, we selected and developed a hairy root line from the peanut cultivar Tainan9 T9-K599. The hairy root line was confirmed by molecular characterization and was subjected to elicitor treatment with MeJA and CD. No report so far has been associated the total antioxidant activity with antioxidant enzyme gene expression and in vitro anti-inflammatory activity in the peanut hairy root treated with MeJA and CD. We therefore investigated the antioxidant activity as well as antioxidant enzyme gene expression, the total phenolic compound, resveratrol and stilbene derivative contents of elicited hairy roots compared to non-elicited controls. The stilbene compound profile of elicited hairy roots was identified by LC-MS/MS. The information obtained from this promising hairy root line may contribute to a potent biological model for enhancing the production of potentially active molecules.

Materials and methods

Plant material

Seeds of peanut (*Arachis hypogea*) cultivar Tainan9 were surface sterilized with 15% Clorox bleach for 10 min and then washed three times with sterile water. The embryos of sterile seeds were separated and germinated on ½ MS (Murashige and Skoog 1962) medium supplemented with 30 g/l sucrose and 7.5 g/l agar, pH 5.7. The germinated seedlings were grown under a 16-h photoperiod at 25–26 °C and 50–60% relative humidity in a plant tissue culture room. At 2 weeks, healthy peanut seedlings with true leaves were further utilized for *Agrobacterium* transformation.

Peanut transformation with *Agrobacterium rhizogenes*

The three wild-type strains of *A. rhizogenes*; K599 (NCPPB 2659), TISTR 511 (ATCC11325), and TISTR 509 (ATCC13332), were used for peanut transformation. All *A. rhizogenes* strains were kindly provided by Assoc. Prof. Dr. Sermsiri Chanprame (Faculty of Agriculture, Kamphaeng Saen, Kasetsart University, Thailand). Each strain of *A. rhizogenes* was cultured in liquid LB medium at 28 °C to log phase (OD600 = 0.5–0.8). Whole peanut seedlings and explants of different organs, such as the leaf, petiole, and hypocotyl, were wounded with a scalpel and then co-cultivated with suspension cultures of each of the three *A. rhizogenes* strains containing 50 µM acetosyringone for 30 min with continuous shaking. Infected seedlings were subsequently transferred to ½MS agar medium containing 50 µM acetosyringone and incubated in the dark at 25–26 °C for 3 days. Excess *A. rhizogenes* was removed by washing infected seedlings with sterile water containing cefotaxime (250 mg/l) and carbenicillin (250 mg/l). The explants were transferred to MS agar medium containing 3% sucrose, 0.75% agar and antibiotic cefotaxime (250 mg/l) and carbenicillin (250 mg/l). All cultures were incubated under a 16-h photoperiod. Emerging roots from explants were observed after 1–2 months, and individual roots were excised and cultured for root multiplication on antibiotic-free MS agar medium.

Identification of peanut hairy roots by PCR

The transformed hairy roots were confirmed by PCR analysis using the specific primers *rolB* and *rolC*, and *Agrobacterium* contamination was detected using the *virD2* primer. Genomic DNA extraction from the selected hairy root line (T9-K599) was performed using the innuPREP plant DNA extraction Kit (Analytik Jena, Germany). Primers rolB-F: 5′-ATTTCCAGAAAAGGTGGGCC-3′ and rolB-R: 5′-GTGATGCCGCAAGCAATGAC-3′ were designed to amplify a product with a size of 495 bp. Primers rolC-F: 5′-CGTCCAGCGATGAGCTAAAG-3′ and rolC-R: 5′-GCTGGTTGCTGGCATAAAGG-3′ were designed to amplify a 480 bp product from the *rolC* gene (Laksana 2008). The highly conserved *virD2* region of *Agrobacterium* was amplified using primers virD2-F: 5′-ATGCCCGATCGAGCTCAAGT-3′ and virD2-R: 5′-CCTGACCCAAACATCTCGGCTGCCCA-3′ with a PCR product of 338 bp (Haas et al. 1995). Amplification conditions for *rolB* and *rolC* gene were initiated by denaturation at 95 °C for 5 min followed by 33 cycles of denaturing at 95 °C for 20 s, annealing at 58 °C for 10 s, extension at 72 °C for 30 s and a final extension period at 72 °C for 7 min. Similar conditions were applied to *virD2* gene amplification, except the annealing temperature was 65 °C.

Growth curve of hairy root culture

Measurement of hairy root growth was carried out in 125-ml Erlenmeyer flasks containing 25 ml of ½ MS medium and inoculated with 0.1 g of hairy roots. The flasks were incubated in the dark at 25 °C in a rotary shaker at 100 rpm. The hairy roots were cultured for 36 days and harvested at day 4, 8, 12, 20, 28 and 36 to measure hairy root fresh weight (FW) and dry weight (DW). The pH and conductivity of the culture medium were recorded, and all experiments were performed in triplicate.

Elicitor treatment with methyl jasmonate and methyl-β-cyclodextrin

The elicitors used in this study were 100 µM methyl jasmonate (MeJA), 6.87 mM methyl-β-cyclodextrin (CD) and a co-treatment with 100 µM MeJA plus 6.87 mM CD as described by (Yang et al. 2015). Root tip tissue (0.5 g) from hairy root line T9-K599 was inoculated into 125-ml Erlenmeyer flasks containing 25 ml of ½ MS medium in a rotary shaker at 25 °C in darkness for 9 days. After 9 days, the hairy roots were moved to fresh ½ MS medium before treatment with each elicitor. The control group was made by replacing the elicitor with EtOH in ½ MS medium. All treatments were performed at 25 °C in the dark for 24 h with three biological replicates.

Hairy root and culture medium extraction

After elicitor treatment, hairy root tissue was collected and subsequently ground to a fine powder using liquid N2, followed by ethyl acetate extraction for 24 h. The culture medium from each treatment was partitioned and extracted twice with ethyl acetate. Ethyl acetate evaporation was carried out on a rotary evaporator (Büchi) at 40 °C, and crude extract was collected. The hairy root tissue and culture medium crude extracts were weighed and resuspended in EtOH to obtain a 10 mg/ml concentration for further analysis.

Total antioxidant assay

Total antioxidant assay was performed using the ABTS assay as described by Re et al. (1999). The ABTS radical cation ($ABTS^{\cdot+}$) was prepared before use by oxidizing a 7 mM ABTS solution with 2.45 mM potassium persulphate in the dark for 12–16 h. The assay reaction was started by adding 2 µl of sample crude extract (10 mg/ml) to 198 µl of the $ABTS^{\cdot+}$ radical. The reaction was incubated for 6 min in

the dark at room temperature, and the absorbance at 734 nm was measured. Trolox was used as the standard, and the antioxidant values are expressed as TEAC (Trolox equivalent antioxidant capacity, mM Trolox/g DW).

Total phenolic content assay

The total phenolic content was measured using the Folin–Ciocalteau method. The reaction was initiated by mixing 2 µl of the sample crude extract (10 mg/ml) with 50 µl of Folin's reagent, followed by the addition of 50 µl of a sodium carbonate (20% w/v) solution. The reaction was incubated for 30 min in the dark, and the absorbance was measured at 765 nm. Gallic acid was used as the standard, and the total phenolic contents are reported as GAE (gallic acid equivalent, µg gallic acid/g DW) value.

High-performance liquid chromatography (HPLC)

HPLC analysis was performed as described by Lee et al. (2004). Separation was performed using a reversed-phase HPLC system with a C18 column (Luna 5 µm C18 (2) 100 A column, Phenomenex). The mobile phase, containing acetonitrile: water (35:65, v/v), was used at a constant flow rate of 1 ml/min. The chromatogram was recorded by using a UV detector at 306 nm. Commercial trans-resveratrol was used to prepare the standard curve for quantitative analysis. The contents of trans-arachidin-1 and trans-arachidin-3 were determined based on the peak area equivalent to the trans-resveratrol standard and expressed as µg resveratrol/g DW.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS)

LC-MS/MS analysis was conducted on a 6540 UHD Accurate–Mass-Q–TOF-LC/MS (Agilent Technologies, Palo Alto, CA, USA). The HPLC column was a reverse-phase Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm (Agilent Technologies, USA). The mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The HPLC conditions used were as follows: at 0 min 95:5 (A:B v v-1), followed by a linear gradient to 5:95 (A:B v v-1) over 30 min, and then holding for 10 min. The column was equilibrated for 5 min between runs before starting a new injection. The column flow rate was set at 0.5 ml/min. The conditions for mass spectrometry were: nebulizer gas pressure 30 psig, gas temperature 350 °C, gas (N2) flow rate 10 l/min, capillary voltage 3500 V, fragmentor potential 100 V, Vcap 3500 V, Skimmer 65 V, and Octopole RFPeak 750 V. Mass spectra were obtained in negative ion (-ESI) mode in the m/z range 100–1000. MS/MS was set up with collision energies of 10, 20, 40 and 100 eV. Data acquisition and analysis were performed using Agilent LC-MS-QTOF MassHunter Data Acquisition Software version B.05.01 and Agilent MassHunter Qualitative Analysis Software B 06.0, respectively (Agilent Technologies, USA).

Lipoxygenase inhibition assay

The lipoxygenase inhibition assay with slight modifications was measured following the method proposed by Tappel (1953). The assay mixture of soybean lipoxygenase enzyme (34,160 U/mL), 10 mM phosphate buffer (pH 8), and various concentrations of peanut hairy root and culture medium crude extract were incubated 10 min at 25 °C. Substrate Linoleic acid (2230 µM) was then added to the reaction mixture to start the enzyme assay reaction and measured the absorbance at 234 nm for 6 min. Nordihydroguaiaretic acid (NDGA) was used as positive controls and ethanol was used as the negative control for lipoxygenase inhibition. The result was expressed as IC50 and the % inhibition was calculated using the equation:

$$\%\,\text{inhibition} = \frac{(\Delta A/\text{min solvent}) - (\Delta A/\text{min sample})}{(\Delta A/\text{min solvent})}$$

RNA extraction and cDNA synthesis

Total RNA was isolated from T9-K599 hairy root tissue using total RNA extraction kit (RBC Bioscience, Taiwan) following the manufacturer's instructions. The purity and quantity of RNA samples were measured using Nanodrop ND-2000 spectrophotometer (Thermo Scientific, USA). One µg of RNA was further converted to cDNA using AccuPower RT-PreMix cDNA synthesis kit (Bioneer, Korea). The cDNA was synthesized with oligo dT primer in a total volume of 20 µl according to the manufacturer's instructions.

Quantitative real time PCR (qPCR)

qPCR primers for antioxidant enzyme and reference genes were designed using NCBI Primer Blast tool. Three selected antioxidant enzyme genes were *CuZn-SOD* (CuZn superoxide dismutase), *GPX* (glutathione peroxidase) and *APX* (ascorbate peroxidase). The *actin* was used as the reference gene in expression analysis. All gene sequences were obtained from *A. hypogaea* available in nucleotide database (GenBank, NCBI) as described in Table 1. The PCR product ranging was 100–150 bp. All primer efficiency was determined with the slope of the standard curve.

qPCR amplification reactions were prepared following the instructions of FastStart Universal SYBR Green Master (Roche, Germany). The total volume of 20 µl reaction mixture containing: 10 µl of 2X SYBR Green Master mix, 1 µl of each forward and reverse primer (0.5 µM final concentration), 1 µl of cDNA and 7 µl of RNase-free distilled water.

Table 1 Primer sequences for antioxidant enzyme and reference genes for qPCR analysis

Gene	Genbank accession no.	Primer sequence	Amplicon (bp)	Tm (°C)
CuZn-SOD	DQ097721.1	F: 5′-GCGGAGTAGCTTGTGGGATT-3′ R: 5′-ACCAAACAACGGAAAGGGGT-3′	131	63
GPX	DQ889534.1	F: 5′-CTCTCTCGGCCTTATCAGCG-3′ R: 5′-CTGGCCATGGTGTGATCTGT-3′	116	60
APX	KC594039.1	F: 5′-CCCCTCATCTTTGACAACTCT-3′ R: 5′-GCATCTTCATCCGCAGCATA-3′	143	60
Actin	DQ873525.1	F: 5′-CCTGGAATCCATGAAACCACA-3′ R: 5′-TCAGCAATGCCTGGGAACAT-3′	119	60

All qPCR reactions and non-template control (NTC) were performed in triplicate using Exicycler™ 96 PCR system (Bioneer, Korea). The amplification reactions were as follow: pre-denaturation step at 95 °C for 10 min, followed by 40 cycles with denaturation at 95 °C for 15 s, annealing at optimum temperature (as described in Table 1) for 10 s, and extension at 72 °C for 10 s. The melting curve analysis was performed after PCR reaction and the threshold cycle (Cq) generated from qPCR system was used for gene expression calculation. The relative quantification of target antioxidant enzyme genes from different elicitor treatment was normalized using *actin* as the reference gene. The results of gene expression fold change were calculated according to $2^{-\Delta\Delta CT}$ method, which addressed to the fold change of target gene expression in treated sample relative to untreated sample, normalized to a reference gene.

Statistical analysis

The experimental data represent the mean ± standard deviation of three replicates per sample. Statistical analysis was performed using one-way analysis of variance (ANOVA) with SPSS software version 17.0. Statistically significant differences were considered at the $P < 0.05$ level.

Results

Establishment of peanut hairy root

Initiation of hairy roots on peanut cultivar Tainan9 was observed after 1–2 months, depending on the individual explants. Among the various explants, whole seedlings were the most efficient tissue for hairy root emergence. The *A. rhizogenes* strain K599 (NCPPB 2659) showed efficient *A. rhizogenes* -mediated transformation at the wound site of peanut cultivar Tainan9 explants. Individual emerging roots were excised from explants and subcultured on MS agar medium containing an antibiotic to eliminate *A. rhizogenes* contamination. The phenotype of the selected hairy root lines was characterized by rapid growth with high lateral branching, no visible root hairs on hormone free medium, and plagiotropic growth (Fig. 1a) compared to wild-type roots (Fig. 1b).

Identification of hairy root by PCR

The integration of *rol* genes from the Ri plasmid of *A. rhizogenes* into the plant genome caused the formation of hairy roots. PCR analysis was used to confirm the presence of *rol* genes (*rolB* and *rolC*) in transformed peanut hairy root lines, and the *virD2* gene was used to access *A. rhizogenes* contamination in hairy roots. PCR (Fig. 2) revealed that *rolB* (495 bp) and *rolC* (480 bp) genes were present in hairy root, confirming that the T9-K599 line was transformed. The absence of amplicons from the *virD2* gene (338 bp) indicated that no bacterial contamination was present in this line. Untransformed peanut roots were used as a negative control in the PCR assay.

Growth curve of hairy root cultures

The T9-K599 hairy root line (0.1 g) was cultured in ½ MS liquid medium and monitored for root growth over 36 days (Fig. 1c). As can be seen from the fresh and dry weight data shown in Fig. 1d, three phases of hairy root growth were identified. A lag phase was observed between 0 and 4 days, and an exponential growth phase continued from 4 to 20 days, followed by a stationary phase that started from day 20. The conductivity of the medium decreased with growth, which was presumably caused by the uptake of inorganic substances by cells, and this change in conductivity corresponded directly to the growth rate of the hairy root cultures. The pH of the culture medium was slightly lower during the lag phase and stayed constant over the subsequent growth period. The pH might not be affected by the nutrient uptake and the growth of hairy roots (Fig. 1e).

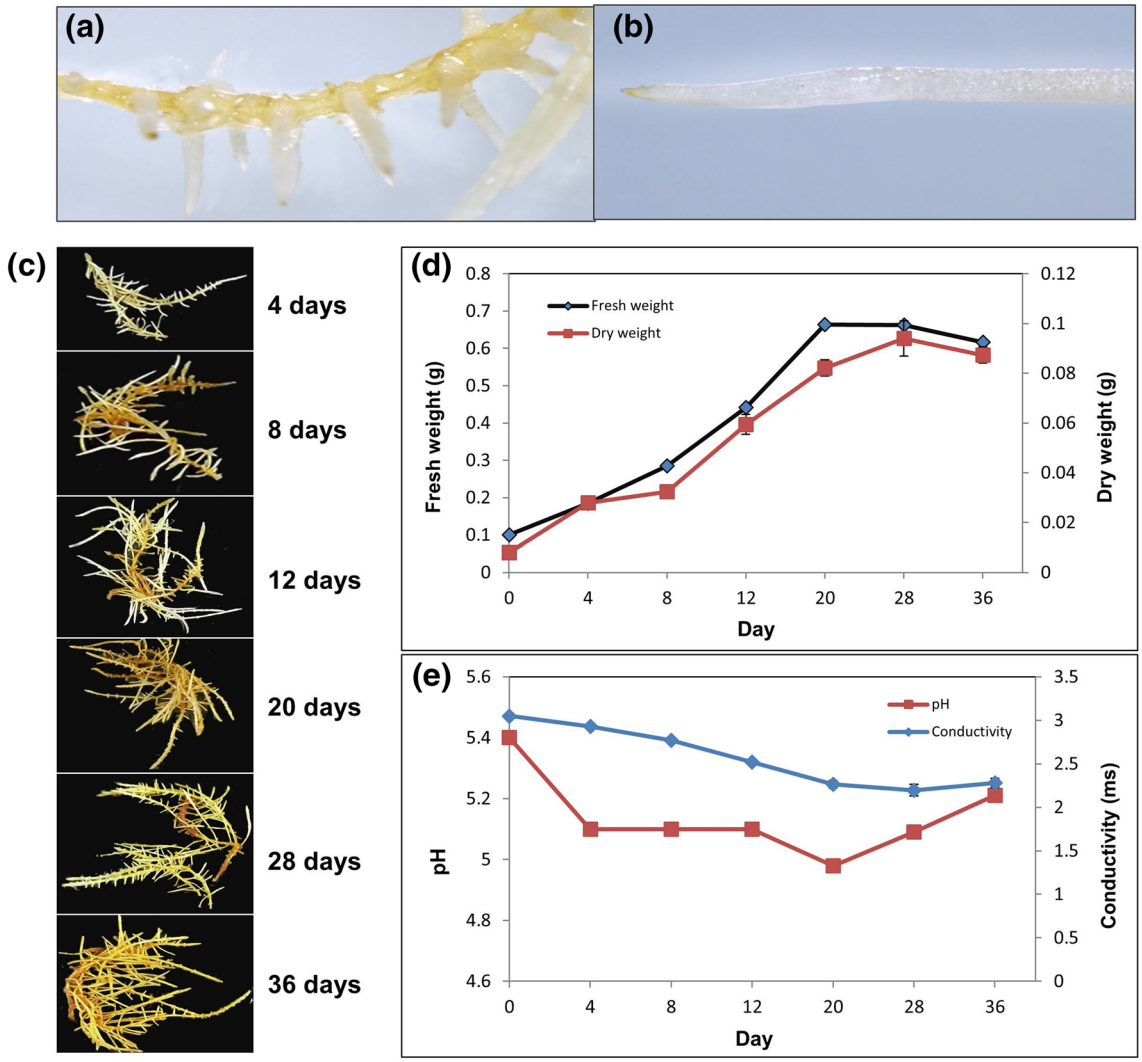

Fig. 1 The phenotype of roots. **a** Hairy root line T9-K599 with high lateral branching and no visible root hairs. **b** Wild-type root from peanut cultivar Tainan 9. **c** Growth of hairy root line T9-K599 at selected time points through 36 days. **d** Growth curve of hairy root line T9-K599 determined by fresh weight and dry weight over 36 days of growth. **e** The pH and conductivity of hairy root culture medium at different stages of growth

Elicitors treatment

Based on the growth of the hairy root line T9-K599, the elicitation experiment was performed during the exponential growth phase with 9-day-old hairy roots. The hairy roots were treated with elicitors for 24 h: 100 µM MeJA, 6.87 mM CD, and co-treatment with 100 µM MeJA and 6.87 mM CD. After 24 h, the medium of the hairy root cultures elicited with co-treatment of MeJA and CD was a yellowish colour that was distinct from that of the other treatment groups.

Antioxidant activity and total phenolic compounds

The antioxidant activity, as measured by the ABTS assay, is presented in Fig. 3a. Interestingly, the antioxidant activity of the culture medium was higher in the group treated with CD (TEAC value 28.30 ± 2.70 mM Trolox/g DW) and group co-treated with MeJA and CD (TEAC value 26.30 ± 1.34 mM Trolox/g DW), which were 23.60- and 22-fold higher than the control group, respectively. The TEAC value of both treatment groups was significantly different from that of the control group. The total phenolic content of the hairy root tissues treated with any of the elicitors was not significantly different from that of the non-elicited control group. The phenolic content of the culture medium was consistent with the antioxidant content results, which showed an increased GAE value in the groups treated with CD and co-treated with MeJA and CD (Fig. 3b). The group co-treated with MeJA and CD exhibited the highest phenolic content of 10.80 ± 1.00 µg gallic acid/g DW, and the CD-elicited

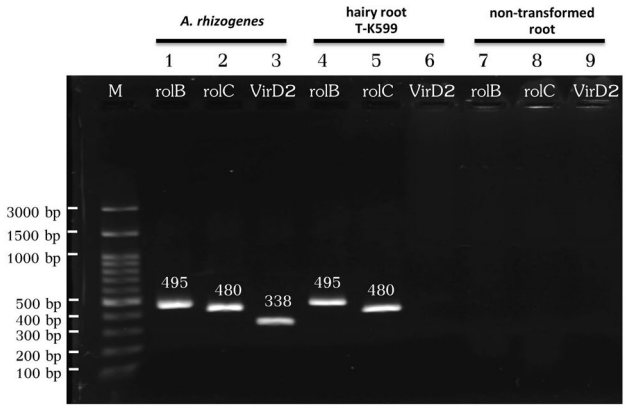

Fig. 2 The PCR of *rolB* (495 bp), *rolC* (480 bp), and *virD2* (338 bp) genes. Lane M; 100 bp DNA ladder marker. Lane 1–3; the positive control of *A. rhizogenes* Ri plasmid. Lane 4–6; transformed hairy root T9-K599 line. Lane 7–9; the non-transformed peanut cultivar Tainan9 root

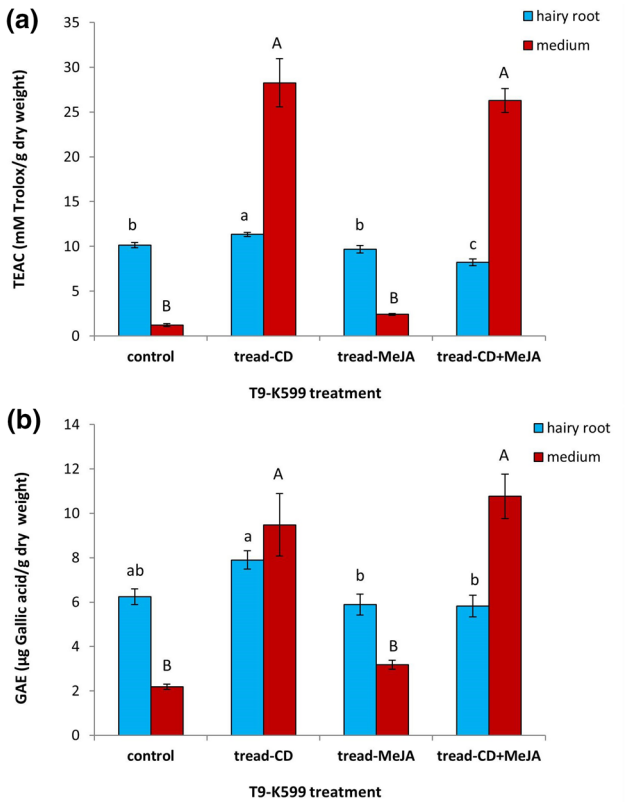

Fig. 3 **a** Antioxidant activity (TEAC value); **b** phenolic content (GAE value) of hairy root T9-K599 and the culture medium crude extract treated with different elicitors; MeJA, CD, and the co-treatment with MeJA and CD. Data represent the mean ± standard deviation of three replicates per sample. Different lowercase letters represent significant differences between different elicitor treatments in hairy root tissue crude extracts ($p < 0.05$). Different capital letters represent significant differences between different elicitor treatments ($p < 0.05$) in hairy root culture medium

group had a phenolic content of 9.50 ± 1.40 µg gallic acid/g DW, which were 4.90- and 4.30-fold higher than the control group, respectively.

Stilbene compounds determination by HPLC

Stilbene compounds, such as resveratrol, trans-arachidin-1, trans-arachidin-3 and unknown stilbene derivatives, were observed in HPLC chromatograms of hairy root culture media treated with CD alone and co-treated with MeJA and CD (Fig. 4). The resveratrol content was calculated according to a commercial trans-resveratrol standard. As shown in Table 2, CD treatment resulted in a culture medium resveratrol content of 64.46 ± 3.48 µg/g DW, whereas the combination of MeJA and CD resulted in resveratrol accumulation of 71.95 ± 3.42 µg/g DW. Because commercial standards for trans-arachidin-1 and trans-arachidin-3 were unavailable, peaks A, and B from the HPLC chromatogram (Fig. 4a, b) were fractionated, purified and identified by MS/MS analysis. As the MS results in Online Resource 1 show, peak A was confirmed to be trans-arachidin-1. The amount of trans-arachidin-1 was calculated based on the peak area equivalent to the trans-resveratrol standard and expressed as µg resveratrol/g DW. The content of trans-arachidin-1 detected in hairy root culture medium treated with CD was 223.81 ± 19.37 µg resveratrol/g DW. Co-treatment with MeJA and CD resulted in lower levels of trans-arachidin-1, at 179.31 ± 10.45 µg resveratrol/g DW (Table 2). Peak B was confirmed to be trans-arachidin-3 by MS/MS spectroscopy (Online Resource 2). The amount of trans-arachidin-3 was also calculated based on the resveratrol equivalent. The content of trans-arachidin-3 in hairy root culture medium treated with CD and co-treated with MeJA and CD were not significantly different, 20.18 ± 3.46 µg resveratrol/g DW and 21.04 ± 0.97 µg resveratrol/g DW, respectively.

Characterization of stilbene compound profiles by LC-MS/MS

The LC-MS/MS method was used to characterize the compounds secreted in the culture medium of hairy roots co-treated with MeJA and CD. Peak identification from full-scan MS and auto MS/MS experiments was determined based on retention times, mass spectra and fragmentation patterns in the negative ion mode based on their m/z charge compared to the reference compound and published data. The LC-MS/MS chromatogram and fragmentation scheme of the culture medium of hairy root T9-K599 treated with MeJA and CD were presented in Fig. 5. As shown in Table 3, peak 1 was tentatively identified as hydroxybenzoic acid with a molecular ion $[M-H]^-$ at m/z 137 and a fragment ion at m/z 108 $[M-H-29(CHO)]^-$ through the loss of the CHO group. Peak 2 gave a precursor ion at

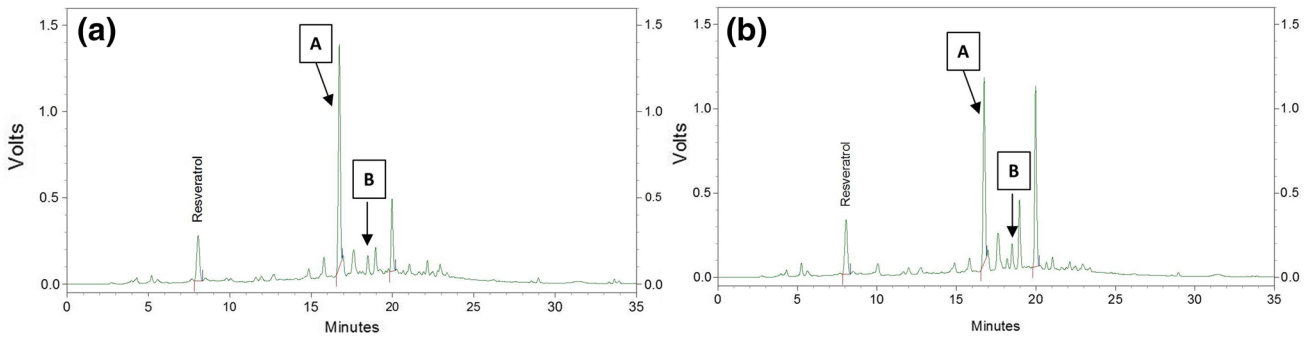

Fig. 4 The HPLC chromatograms of hairy root culture medium treated with different elicitors; **a** CD treatment **b** co-treatment with MeJA and CD

Table 2 Resveratrol, arachidin-1, arachidin-3 content of hairy root culture medium treated with CD alone and co-treated with MeJA and CD

Treatment	Resveratrol (µg/g DW)	Arachidin-1 (µg resveratrol/g DW)	Arachidin-3 (µg resveratrol/g DW)
Control	ND	ND	ND
MeJA	ND	ND	ND
CD	64.46 ± 3.48	223.81 ± 19.37	20.18 ± 3.46
MeJA + CD	71.95 ± 3.42	179.31 ± 10.45	21.04 ± 0.97

Data represent the mean ± standard deviation of three replicates per sample

m/z 179 and was assigned as caffeic acid, with a fragment ion at m/z 135 $[M\text{-}H\text{-}44(CO_2)]^-$ through the loss of the CO_2 group. Peaks 3–14 were tentatively identified as stilbene compounds. Peak 3 had an m/z of 243, followed by dissociation ions at 225 $[M\text{-}H\text{-}18(H_2O)]^-$, 201 [M-H-42 (CHCOH)]$^-$, and 159 $[M\text{-}H\text{-}42(C_2H_2O)\text{-}42(C_2H_2O)]^-$ indicating that this peak corresponds to trans-piceatannol. Peak 4 was identified as trans-resveratrol; the molecular ion was at m/z 227 and cleavage ions were present at m/z 209 $[M\text{-}H\text{-}18(H_2O)]^-$, 185 $[M\text{-}H\text{-}42(C_2H_2O)]^-$, and 143 $[M\text{-}H\text{-}42(C_2H_2O)\text{-}42(C_2H_2O)]^-$. This result confirmed the presence of resveratrol with a molecular ion m/z 227 and exhibiting an identical retention time and m/z value as the commercial trans-resveratrol standard. Peak 5, the cis-resveratrol isomer had a molecular ion at m/z 227 and a fragmentation pattern similar to that observed for trans-resveratrol, with ions at 185 $[M\text{-}H\text{-}42(C_2H_2O)]^-$ and 143 $[M\text{-}H\text{-}42(C_2H_2O)\text{-}42(C_2H_2O)]^-$. However, cis-resveratrol had a longer retention time compared to trans-resveratrol, due to the more hydrophobic nature of the cis-isomer compared to trans-resveratrol. Peaks 6, 7, and 8 were tentatively identified as a trans-arachidin 1 isomer form, with a molecular ion at m/z 311 and fragment ions at m/z 242 and 267 $[M\text{-}H\text{-}44(CO_2)]^-$ through the loss of CO_2 groups. Although these compounds had the same molecular ion, the time at which each isomer molecule eluted from the column was different. Peak 9 was likely 4-Isopentadienyl-3,5,3′,4′-tetrahydroxystilbene (IPP) with a molecular ion at m/z 309 and a product ion at 265 $[M\text{-}H\text{-}44(CO_2)]^-$ with the loss of a CO_2 group. Peaks 10 and 11 were probably trans-arachidin 3 isomer molecules, with a molecular ion at m/z 295 and fragment ions at m/z 239 [M-H-28(CO)-28(CO)]$^-$ and different retention times. Peaks 12 and 14 were likely arahypin-7 isomer molecules, with a molecular ion at m/z 621 and a dissociation fragment of m/z 511 $[M\text{-}H\text{-}42(C_2H_2O)\text{-}68(C_3O_2)]^-$. Peak 13, with a molecular ion at m/z 293 and a fragment ion at m/z 209 $[M\text{-}H\text{-}42(C_2H_2O)\text{-}42(C_2H_2O)]^-$, was tentatively identified as trans-3′-Isopentadienyl-3,5,4′-trihydroxystilbene (IPD).

Anti-inflammatory activity

The lipoxygenase enzyme inhibitory assay was used for in vitro anti-inflammatory determination. The enzyme assay was evaluated using a kinetic spectrophotometric assay with absorbance at 234 nm. Various concentrations of peanut hairy root and medium culture crude extracts treated with different elicitors were tested for inhibitory activity and the results were presented as IC50 values in Fig. 6. NDGA was chosen for positive control, which exhibited low IC50 values of 0.0004 mg/ml. Hairy root culture crude extract represented the higher IC50 compared to medium culture crude extract. The low value of IC50 among the medium culture crude extract represented the excretion of bioactive compound into the culture medium. The culture medium elicited with CD alone and combination of MeJA plus CD had potentially high lipoxygenase enzyme inhibition activity with low IC50 value of 0.0065 mg/ml and 0.0095 mg/ml, respectively. This result indicated the promising anti-inflammatory activity of medium culture from CD and combination of MeJA plus CD treated peanut hairy root.

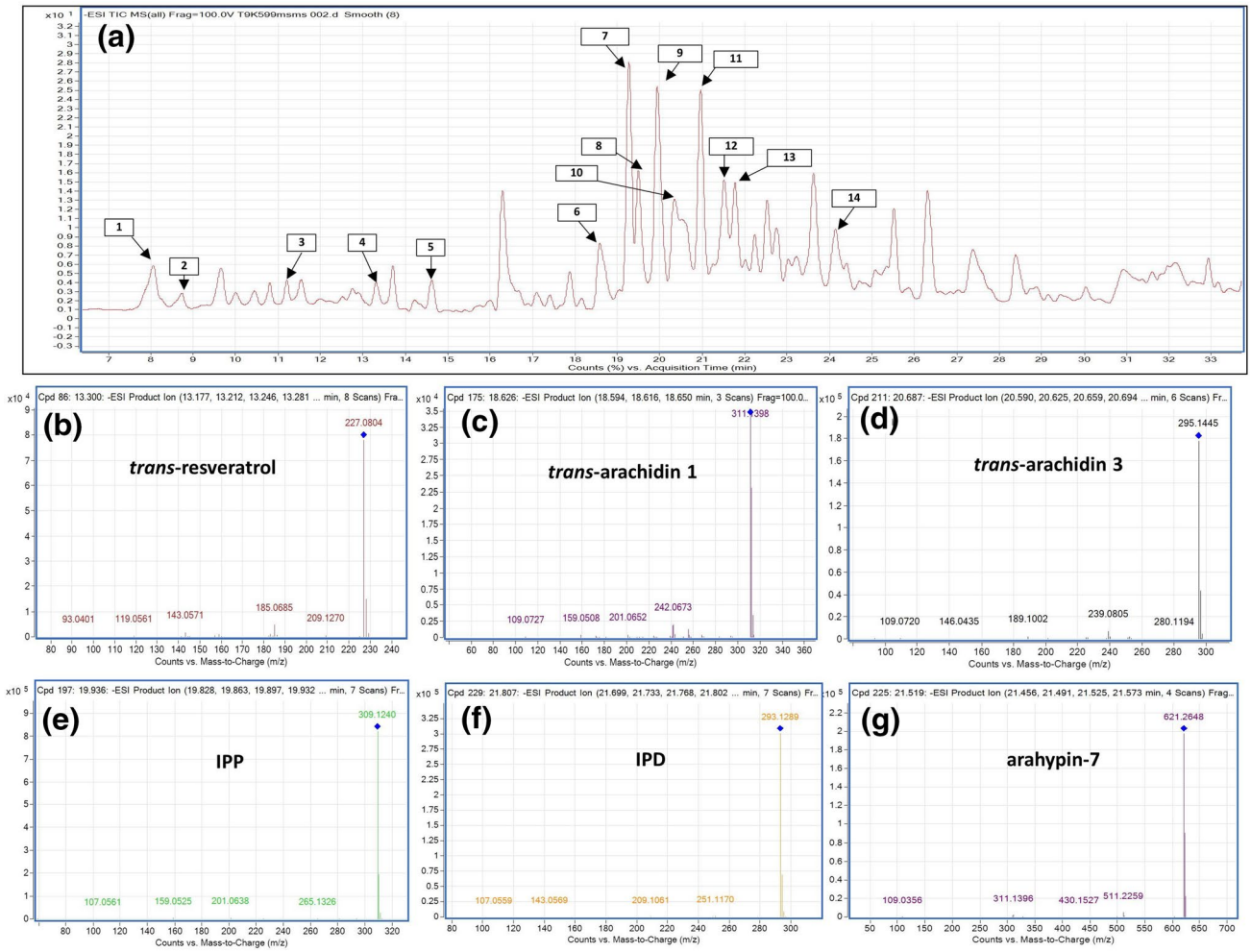

Fig. 5 LC-MS/MS spectra of the hairy root culture medium crude extract co-treatment with MeJA and CD. **a** The total ion current (TIC) output from the ESI-MS in negative mode. The peak labels in the chromatogram represent the peak number. Tentative identification from MS/MS-spectra of **b** trans-resveratrol, **c** trans-arachidin-1, **d** trans-arachidin-3, (**e**) IPP, (**f**) IPD, (**g**) arahypin-7

Antioxidant enzyme gene expression profile

The antioxidant enzyme (*CuZn-SOD, APX, GPX*) gene expression profiles were performed in hairy root culture treated with MeJA, CD and a co-treatment with MeJA plus CD compared to non-elicited control for 24 h. As the result shown in Fig. 7, the *CuZn-SOD* gene was significantly up-regulated in the treatment with CD alone which exhibited 1.41 fold change compared to the control group. Interestingly, the *CuZn-SOD* gene expression was down-regulated in the treatment with MeJA alone and co-treatment of MeJA together with CD sample which was 0.13 and 0.20 fold change compared to non-elicited control. The similar trend was observed in the *APX* gene expression which was induced in the CD treated hairy root (fold change 1.38). The low level *APX* gene expression was detected in the MeJA treated sample and co-treatment of MeJA and CD with 0.11 and 0.14 fold change. The expression level of GPX gene was not significant different in CD alone and MeJA alone treated group but decreased to 0.57 fold change in the co-treatment with MeJA plus CD group.

Discussion

In this study, the hairy root phenotype was characterized by rapid and vigorous plagiotropic growth with extensive lateral branching and no root hairs. Molecular characterization confirmed the transformation of the *rolB* and *rolC* genes from *A. rhizogenes* into the peanut genome. No detectable *virD2* gene was observed in T9-K599 hairy roots, indicating no contamination by *A. rhizogenes*. The absence of root hairs is a typical characteristic and was also observed in peanut hairy roots, as reported by Medina-Bolivar et al. (2007), with high lateral branching along the central root. Akasaka et al. (1998) demonstrated by histological observation that transformed

Table 3 LC-MS/MS data from the hairy root culture medium co-treatment with MeJA and CD

Peak	t_R (min)	MW	$[M-H]^-$ (*m/z*)	MS/MS (*m/z*)	Proposed compound
1	7.9	138	137	119, **108**	Hydroxybenzoic acid
2	8.6	180	179	151, **135**, 125, 113	Caffeic acid
3	11.4	244	243	**225, 201**, 175, **159**	Trans-piceatannol
4	13.3	228	227	**209, 185, 143**, 119, 93	Trans-resveratrol
5	14.7	228	227	**185, 143**, 107, 65	Cis-resveratrol
6	18.6	312	311	**242**, 201, 159, 109	Trans-arachidin 1-isomer1
7	19.2	312	311	**242**, 201, 159, 109	Trans-arachidin 1-isomer2
8	19.5	312	311	295, **267**, 239, 159, 109, 67	Trans-arachidin 1-isomer3
9	19.9	310	309	**265**, 201, 159, 107	4-Isopentadienyl-3,5,3′,4′-tetrahydroxystilbene (IPP)
10	20.6	296	295	280, **239**, 189, 146, 109	Trans-arachidin 3-isomer1
11	20.9	296	295	267, **239**, 185, 157	Trans-arachidin 3-isomer2
12	21.5	622	621	**511**, 430, 311, 109	Arahypin-7-isomer1
13	21.8	294	293	251, **209**, 143, 107	Trans-3′-Isopentadienyl-3,5,4′-trihydroxystilbene (IPD)
14	24.3	622	621	499, 377, 311, 265, 199, 109	Arahypin-7-isomer2

The most abundant ions observed in mass spectra (m/z) are shown in bold

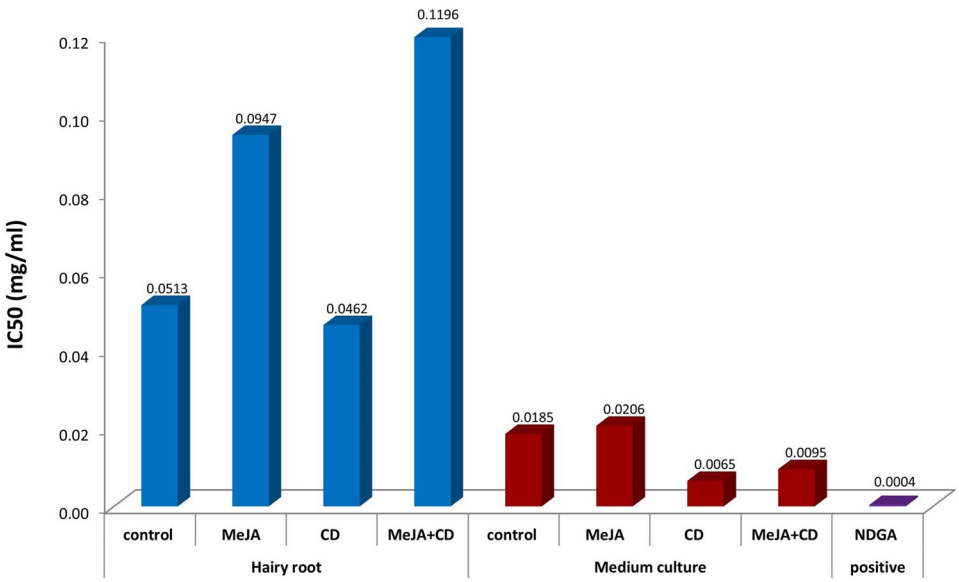

Fig. 6 Anti-inflammatory activity of peanut hairy root and medium culture. Data represent the inhibitory activity (IC50) of crude extract. NDGA was used as positive control

peanut roots exhibited developed epidermal cells, a cortex and a central stele delimited by an endodermis. There are several factors that influence the efficiency of *A. rhizogenes* hairy root transformation, such as the *A. rhizogenes* strain, plant genotype, co-culture conditions (Opabode 2006), the antibiotic selection dosage and antibiotic used to eliminate residual *A. rhizogenes* (Rizvi et al. 2015). The competence of *A. rhizogenes* to infect a particular tissue or plant genotype is relevant to the transformation success. Halder and Jha (2016) evaluated the induction of peanut cv. JL-24 hairy root for trans-resveratrol production and found that *A. rhizogenes* strain LBA9402 showed maximum efficient transformation among the five different strains; LBA9402, A4, R1000, ATCC 15834 and HRI. To evaluate the transformation ability of different *A. rhizogenes* strains in this study, three wild-type strains of *A. rhizogenes*, K599 (NCPPB 2659), TISTR 511 (ATCC11325), and TISTR 509 (ATCC13332), were tested for peanut transformation. The transformation efficiency was different with different *A. rhizogenes* strains, and the highest transformation rate observed in this study was achieved with strain K599 (NCPPB 2659), which produces cucumopine as its major opine. Hsieh et al. (2017) reported the rapid and efficient method to obtain direct shoot organogenesis from peanut cotyledonary node explants which can

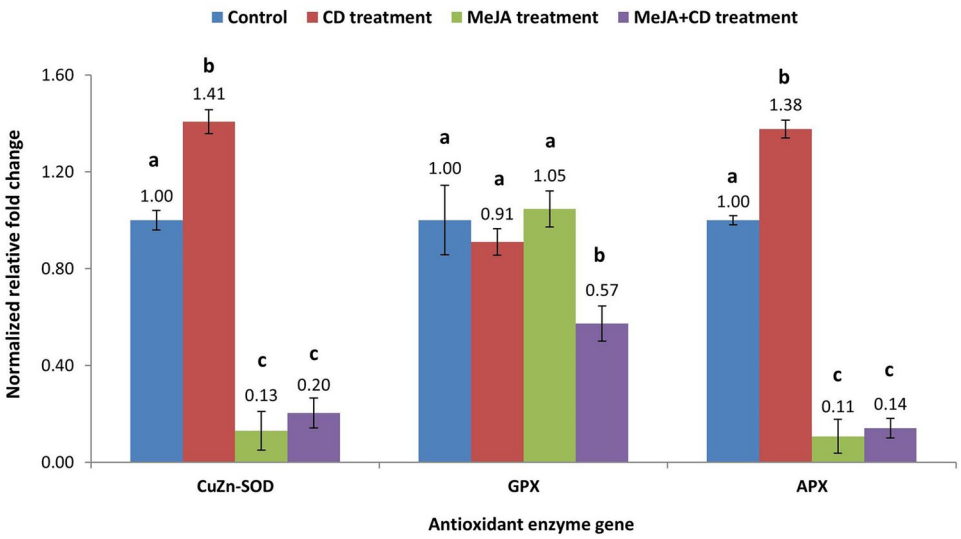

Fig. 7 Gene expression profile of *CuZn-SOD, GPX* and *APX* antioxidant enzyme of peanut hairy root treated with different elicitors; MeJA, CD, and the co-treatment with MeJA plus CD

be used for Agrobacterium-mediated transformation. Our result demonstrated that whole peanut seedlings were the most susceptible to hairy root emergence compared to leaf, petiole, and hypocotyl explants. The success of *A. rhizogenes* transformation could be due to the particular virulence factors of various *A. rhizogenes* strains and host specificity, the relationship between the infection site and the bacterial induction of chemotaxis compounds, and the components of the plant cell wall relating to the initial binding of bacteria to the plant cell before gene transfer occurs (Porter and Flores 1991). Hairy root cultures are considered to be a promising system for the sustainable production of valuable secondary metabolites. The elicitor treatment strategy is predominantly considered to be an efficient approach for secondary metabolite production. Several elicitors, such as methyl jasmonate (MeJA), salicylic acid (SA), H_2O_2, sodium acetate (NaOAc), and cyclodextrin (CD), have been used in the hairy root culture system to enhance bioactive compounds. The combined treatment of UV-B irradiation and MeJA has been shown to have synergistic effects on the expression of genes in the tanshinone biosynthetic pathway and thus enhance the bioactive diterpenoids tanshinones in *Salvia miltiorrhiza* hairy root cultures (Wang et al. 2016). Muscadine grape (*Vitis rotundifolia*) hairy root cultures produced stilbenoids, such as resveratrol, piceid, and ε-viniferin, upon treatment with MeJA. Those stilbenoids were analysed for radical scavenging capacity and were shown to be strong antioxidant bioactive compounds (Nopo-Olazabal et al. 2013). In the present study, the T9-K599 peanut hairy root line was treated with 100 µM MeJA, 6.87 mM CD and a combination of both 100 µM MeJA and 6.87 mM CD. The concentration of both elicitors was chosen in accordance to the report of Yang et al. (2015) which showed the high level of stilbene compound production after treatment of both elicitors. The total antioxidant activity and phenolic contents of hairy root and culture medium crude extracts were examined. After 24 h of treatment, the culture medium treated with CD exhibited 23.60-fold greater antioxidant activity and 4.30-fold higher phenolic content than the control group. This is in accordance with the antioxidant enzyme gene expression result which exhibited the up-regulation of *CuZn-SOD* and *APX* gene expression to 1.41 fold change and 1.38 fold change compared to control, respectively. CD might play an effective role in the regulation of multiple transcriptional pathways which were involved in oxidative stress responses. SOD is a major antioxidant enzyme system to rapidly convert excess $O_2^{\cdot-}$ into H_2O_2. The excess toxic H_2O_2 can subsequently be reduced by H_2O_2 scavenging enzymes, such as CAT, APX and GPX. These enzymes work in concert with the production of antioxidant bioactive compounds to control the excess of ROS and protect cells from ROS oxidative damage (Gill and Tuteja 2010). On the other hand, the MeJA treatment did not show the increasing of total antioxidant and phenolic compounds in the culture medium. Likewise the *CuZn-SOD* and *APX* gene expression result was down regulation due to MeJA treatment. The gene expression of *GPX* was not affected by MeJA treatment as compared to CuZn-*SOD* and *APX* enzyme. The plant GPX was suggested to be more efficient in reducing peroxide substances such as organic hydroperoxides and lipid peroxides than H_2O_2. The exact mechanisms of plant GPX are not yet known but GPX was supposed to contribute to cross talk between different signalling pathways (Bela et al. 2015). Interestingly, the co-treatment of MeJA and CD showed 22.0-fold higher total antioxidant activity and 4.90-fold higher phenolic content than the control group while the CuZn-*SOD, GPX* and *APX* antioxidant enzyme gene expression were down-regulated. This indicates that MeJA mediated suppression of CD-responsive antioxidant enzyme gene expression, thus resulting in the down regulation of a set of antioxidant enzyme

gene. The combination of MeJA and CD might involve in the different intracellular signalling pathway for both elicitors. Almagro et al. (2012) demonstrated that CD alone or combination of MeJA and CD promoted the production of H_2O_2 in the cell with different cross-communicated signalling pathway. In the presence of both elicitors, CD was reported to neutralize the strong oxidative and nitrosative bursts activated by MeJA, and therefore they coordinated both H_2O_2 and NO levels. The result indicated that CD and the combination of MeJA plus CD are promising elicitors to stimulate hairy roots to produce and release potent phenolic compounds with high antioxidant capacity to the culture medium.

In addition to the antioxidant and total phenolic compounds, we identified stilbenes, such as resveratrol, trans-arachidin-1 and trans-arachidin-3, using an HPLC method. The compound profile of stilbenes secreted in the culture medium of hairy roots co-treated with MeJA and CD was also characterized by LC-MS/MS. Stilbene compounds have been reported to be produced by a variety of plants as a defensive response to exogenous stimuli, such as biotic and abiotic stresses (Hasan et al. 2013). A significant increase in trans-resveratrol and resveratrol dimers and oligomers was induced by *Aspergillus carbonarius* infection in Italian grape cv. Negro Amaro (Flamini et al. 2016). Upon challenging peanut seeds with the fungal strain *Rhizopus oligoporus*, the new stilbene derivative (3,5,3′-trihydroxy-4′-methoxy-5′-isopentenylstilbene), new stilbene dimers (arahypin-11 and arahypin-12) and three known stilbenoids (arachidin-1, arachidin-3, and SB-1) were isolated from black skin peanut seeds (Liu et al. 2013). HPLC analysis of elicitor-treated hairy roots in this study is consistent with the antioxidant and total phenolic content. Resveratrol and stilbene derivatives, such as arachidin-1 and arachidin-3, were observed from hairy root medium culture treated with CD alone and the combination of MeJA and CD. The highest resveratrol content (71.95 ± 3.42 µg/g DW) was detected in the culture medium after treating hairy roots with both MeJA and CD. In addition to resveratrol, the other stilbene derivatives were also highly induced after MeJA and CD combined treatment. This result is consistent with the report by Yang et al. (2015), who showed accumulation of high resveratrol, piceatannol, arachidin-1, and arachidin-3 in the culture medium of hairy root cultures (cultivar Hull) after co-treatment with MeJA and CD. The research of peanut hairy root elicited with MeJA and CD available so far has been addressed on some stilbene substances such as resveratrol, piceatannol, arachidin-1, and arachidin-3 (Medina-Bolivar et al. 2007; Condori et al. 2010; Yang et al. 2015). Our present study investigated the induction of several other stilbene compounds in the culture medium of hairy roots after treatment with MeJA and CD using full scan LC-MS and auto MS/MS experiments. The identification of trans-piceatannol, trans-resveratrol, cis-resveratrol, trans-arachidin 1, trans-arachidin 3, IPP, IPD and arahypin-7 were tentatively identified by mass spectra and MS/MS fragmentation patterns in the negative ion mode. The similar MS/MS fragmentation of trans-resveratrol and trans-piceatanol was also reported by Ma et al. (2014). The MS/MS fragmentation patterns of the other stilbene compounds have been reported previously in peanut seeds elicited by *Aspergillus caelatus* (Aisyah et al. 2015).

MeJA is a well-known elicitor and has been accepted as a signal transduction elicitor for plant defence responses (Franceschi et al. 2002). It has been reported that MeJA significantly increases the release of resveratrol and stilbenoids in hairy root cultures of muscadine grape (*Vitis rotundifolia Michx)* (Nopo-Olazabal et al. 2014). However, we did not detect resveratrol and stilbene derivatives in the culture medium of T9-K599 hairy roots treated with MeJA alone. In addition, elicitation with MeJA alone did not activate the accumulation of bioactive antioxidant capacity in the culture medium. Additionally, a low phenolic content and total antioxidant activity were found in the culture medium treated with MeJA alone. Interestingly, Yang et al. (2015) reported the expression of the resveratrol synthase gene was rapidly increase after 3 h of MeJA treatment in peanut hairy roots, indicating that resveratrol might be produced after MeJA elicitation. However, the lack of detectable resveratrol content in the MeJA-treated sample could be due to the poor stability and spontaneous oxidation of resveratrol (Francioso et al. 2014) or the degradation of resveratrol by some specific enzyme, such as the hydroxystilbene-degrading enzyme, which is expressed under some stress conditions (Sbaghi et al. 1996). It is also worth noting that resveratrol degradation can be increased by unfavourable conditions, such as alkaline pH, light and high temperature (Zupančič et al. 2015). CD is composed of cyclic oligosaccharides and forms a hydrophobic cavity in a cone-shape structure. CD has been considered to be a true elicitor. In *Vitis vinifera*, CD activated a signal transduction cascade through several transcription factors to regulate resveratrol biosynthetic gene expression (Bru et al. 2006). In addition, CD can form inclusion complexes with nonpolar molecules inside the hydrophobic cavity (Lu et al. 2009). Co-treatment with MeJA and CD might maintain the resveratrol and stilbene derivative contents due to the capacity of CD to form inclusion complexes with stilbene compounds. It has been shown that the overexpression of Vitis stilbene synthase1 (Vst1) gene in grapevine (*Vitis vinifera* L.) increased the resveratrol content and the resistance to *Botrytis cinerea* compared to control (Dabauza et al. 2015). Several reports have confirmed that the combined treatment of MeJA and CD significantly induced the expression of stilbene biosynthetic genes, thus indicating a synergistic effect of MeJA and CD on resveratrol and stilbene compound production, resulting

in a higher yield of resveratrol when compared with MeJA or CD treatment alone (Lijavetzky et al. 2008; Yang et al. 2015). Recently, the mechanisms of protein phosphorylation and dephosphorylation through mitogen-activated kinase and tyrosine phosphatase pathways have been reported to be involved in the signal transduction pathway triggered by combination of MeJA and CD (Belchí-Navarro et al. 2013).

Lipoxygenase enzyme is one of the key enzymes involved in the regulation of chronic inflammation and oxidative stress generation. The lipoxygenase enzyme activity inhibition assay was used for in vitro anti-inflammatory determination. This is the first report to correlate the in vitro lipoxygenase inhibition assay with the peanut hairy root and medium culture crude extract. In the present study, the medium culture crude extract exhibited the lower IC50 compared to hairy root crude extract. Our previous study demonstrated that lipoxygenase activity was inhibited by different parts of peanut sprout crude extract, especially the seed coat and root extracts. The highest inhibitory effect was obtained from the seed coat of ungerminated peanut crude extract with IC50 value of 0.01 mg/ml (Limmongkon et al. 2017). The current report of hairy root medium culture crude extract elicited by CD alone and the combination of MeJA and CD showed the lower IC50 value of 0.0065 and 0.0095 mg/ml, respectively. This could be due to the high amount of chemical constituents, especially stilbene compounds production in the hairy root medium culture after elicited by CD alone and the combination of MeJA and CD. The low IC50 value from lipoxygenase inhibition assay of medium culture crude extract elicited by CD alone and co-treatment of MeJA plus CD suggested a relationship of in vitro anti-inflammatory activity, high antioxidant activity, total phenolic compound and stilbene contents. This indicated that antioxidant stilbene compounds may act as anti-inflammatory agents by scavenging ROS. Thus, compounds obtained from peanut hairy root culture extract elicited by different elicitors may be the interested source for bioactive substances production and could serve as promising agents for potent anti-inflammatory treatments.

Conclusion

The peanut hairy root culture line T9-K599, which was established by *A. rhizogenes* transformation of peanut cultivar Tainan9, is a powerful and sustainable source of phenolic compounds with high antioxidant activity. Much research has been focused on the health benefits of phytochemicals, especially the antioxidant properties of phenolic compounds. Stilbenes are a group of polyphenolic compounds produced by several plant species upon various biotic and abiotic stresses. Stilbene compounds have significant beneficial effects to human health due to their powerful antioxidant, anti-inflammatory, and anti-carcinogenic properties. In the present study, the elicitor treatment strategy was used to induce the production of stilbene compounds. Resveratrol, arachidin-1, arachidin-3, IPP, IPD and arahypin-7 were induced and secreted into the culture medium after elicitation of hairy root line T9-K599 with CD alone and co-treatment with MeJA plus CD. Examination of the medium culture also showed increased production of phenolic compounds with high antioxidant activities and *in vitro* anti-inflammatory activity. The scale-up of hairy root culture for mass production and exploration of effective elicitor treatments should be further investigated for enhancement of high-value stilbene bioactive compound production.

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Author contributions VP conducted the molecular characterization, elicitor treatment and HPLC analysis. TS provided the transformation experiment, LC-MS/MS identification and in vitro anti-inflammatory assay. PT and PK contributed to hairy root growth and culture experiment. TW and AK contributed to experiment design and data analysis. AL contributed to experiment design, monitored the experiment progress and wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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