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The induction of antioxidant compound production in cadmium treated peanut hairy root culture

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Abstract

Peanut (*Arachis hypogaea*) produces stilbene compounds with high diverse biological activities such as antioxidant, anti-inflammatory and anticancer. Peanut hairy roots established from peanut cultivar Kalasin2 transformed with *Agrobacterium rhizogenes* K599 (K2-K599) were elicited with various elicitors to induce the antioxidant production. The optimum concentration of elicitors used in the experiment was 5 ppm cadmium (Cd), 100 µM methyl jasmonate (MeJA) and 6.87 mM cyclodextrin (CD). The antioxidant activities were determined using DPPH and FRAP assays. The result showed that the culture medium crude extract of peanut hairy root elicited with co-treatment of MeJA and CD followed by Cd for 48 h exhibited the highest antioxidant capacity with 128.6±1.69 mM Trolox/g DW and 462.43±2.76 mg ascorbic acid/g DW for DPPH and FRAP assays, respectively. This would be the promising strategy for elicitor treatment to enhance the bioactive antioxidant compounds in hairy root culture.

Introduction

Oxidative stress has been reported to be associated with several diseases, such as cardiovascular diseases, aging and cancer[1]. The imbalance between reactive oxygen species (ROS) generation and the antioxidant defense in the cell undergo the oxidative stress. The mechanisms to regulate the ROS in plant are enzymatic antioxidant and non-enzymatic antioxidant defense systems. Non-enzymatic antioxidants are molecules that inhibit the free radical molecules such as plant polyphenols, carotenoids, and glutathione.

Dean et al.[2] reported that crude extract from peanut root exhibited the high antioxidant activity. In addition, the bioactive stilbene compound such as resveratrol has been found to increase by external stimuli in peanut plant[3]. Resveratrol has been shown to possess several beneficial biological effects such as antioxidant, anti-inflammatory, and anti-cancer[4]. Several studies reported that hairy root culture of peanut effectively produced resveratrol and its derivatives[5, 6]. Furthermore, the elicitor treatment is considered the good approach to enhance plant bioactive compounds production. The co-treatment of MeJA and CD has been reported to be a good elicitor to induce the resveratrol and stilbene derivatives in peanut hairy root cultures[6]. Cd has been reported to induce high level of bioactive compounds with antioxidant activity and total phenolic compounds in blueberry plantlets in consequence of Cd-induced oxidative stress[7].

The purpose of this work was to determine the antioxidant production in peanut hairy root culture (K2-K599) induced by co-treatment of MeJA with CD and Cd.

Methodology

Plant material

All experiments were conducted with hairy root cultures of peanut (*Arachis hypogaea*) cultivar Kalasin2 (K2-K599).

Optimum concentration of elicitors

The different concentrations of Cd (1, 5, 10 and 50 ppm) were examined for the optimum concentration of Cd to be used together with co-treatment of 100 µM MeJA plus 6.87 mM CD as described by Yang et al.[6]. All elicitor treatments were performed with hairy root culture in ½ MS medium at 25°C under continuous darkness for 24 h. The culture medium was partitioned and extracted with ethyl acetate. The stilbene compounds were analyzed by HPLC method which performed as described by Limmongkon et al.[8]. The HPLC separation was performed on a C18 reverse-phase column with a flow rate at 1 ml/min. The mobile phase consisted of acetonitrile and 2% formic acid in water. Chromatograms were established using absorbance at 306 nm.

Elicitor Treatments

The four different elicitor strategies were examined as following: 5 ppm Cd; 5 ppm Cd followed by a co-treatment with 100 µM MeJA plus 6.87 mM CD; co-treatment with 100 µM MeJA plus 6.87 mM CD; and co-treatment with 100 µM MeJA plus 6.87 mM CD followed by 5 ppm Cd. All elicitor treatments were performed with hairy root culture in ½ MS medium at 25°C under continuous darkness for 24, 48 and 72 h with three biological replicates.

Hairy root and culture medium extraction

Hairy root tissue was pulverized with liquid nitrogen, followed by ethyl acetate extraction. The culture medium was partitioned and extracted with ethyl acetate. The ethyl acetate fractions were evaporated under vacuum at 40°C in a rotary evaporator. Extracts were dissolved in ethanol for further analysis.

The antioxidant DPPH assay

The free radical scavenging activity was evaluated using the radical DPPH (2,2 diphenyl-1-picrylhydrazyl) technique as described by Brand-Williams et al.[9]. Briefly, 10 µl of crude extract was added to 100 µl of DPPH radical with 90 µl of ethanol and incubated in the dark at room temperature for 30 min. The absorbance was measured at 515 nm. Trolox was used as the standard and the antioxidant was expressed as TEAC (Trolox equivalent antioxidant capacity, mM Trolox/g DW).

The antioxidant FRAP assay

The FRAP (ferric reducing antioxidant power) procedure was determined according to Benzie and Strain[10]. The assay reaction was started by adding 2 µl of crude extract to 198 µl of FRAP reagent (10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3$ in 300 mM acetate buffer pH 3.6), incubated at room temperature for 5 min and measured at 593 nm. The ascorbic acid was used as the standard and the FRAP activity was expressed as ascorbic acid equivalent; AAE (mg ascorbic acid/g DW).

Results and Discussion

Effect of difference elicitors on peanut hairy root culture

Hairy root treated with combination of MeJA and CD and the combination of MeJA and CD together with Cd exhibited a yellowish color in both hairy root tissue and culture medium (Fig. 1a-1b). This might be due to the bioactive compounds production in the treated hairy root culture as described by Yang et al.[6]. Plant produced secondary metabolites for

defensive mechanism against pathogens and other physical stresses. It has been reported that some plant tissue cultures accumulated intracellular secondary metabolites but some released secondary metabolites into the culture medium[11]. Shilpa et al.[12], suggested that the accumulation of intracellular secondary metabolites might inhibit the synthesis of metabolic product through feedback inhibition. Thus the secretion of secondary metabolites into the culture medium could precede cell metabolic pathway in the forward direction. Our previous result showed that peanut hairy root treated with combination of MeJA and CD exhibited higher antioxidant activities in the culture medium crude extract than the hairy root tissue crude extract indicating that some antioxidant substances were released into the culture medium[13]. Therefore, the stilbene compounds were examined in the culture medium crude extract after elicitor treatment.

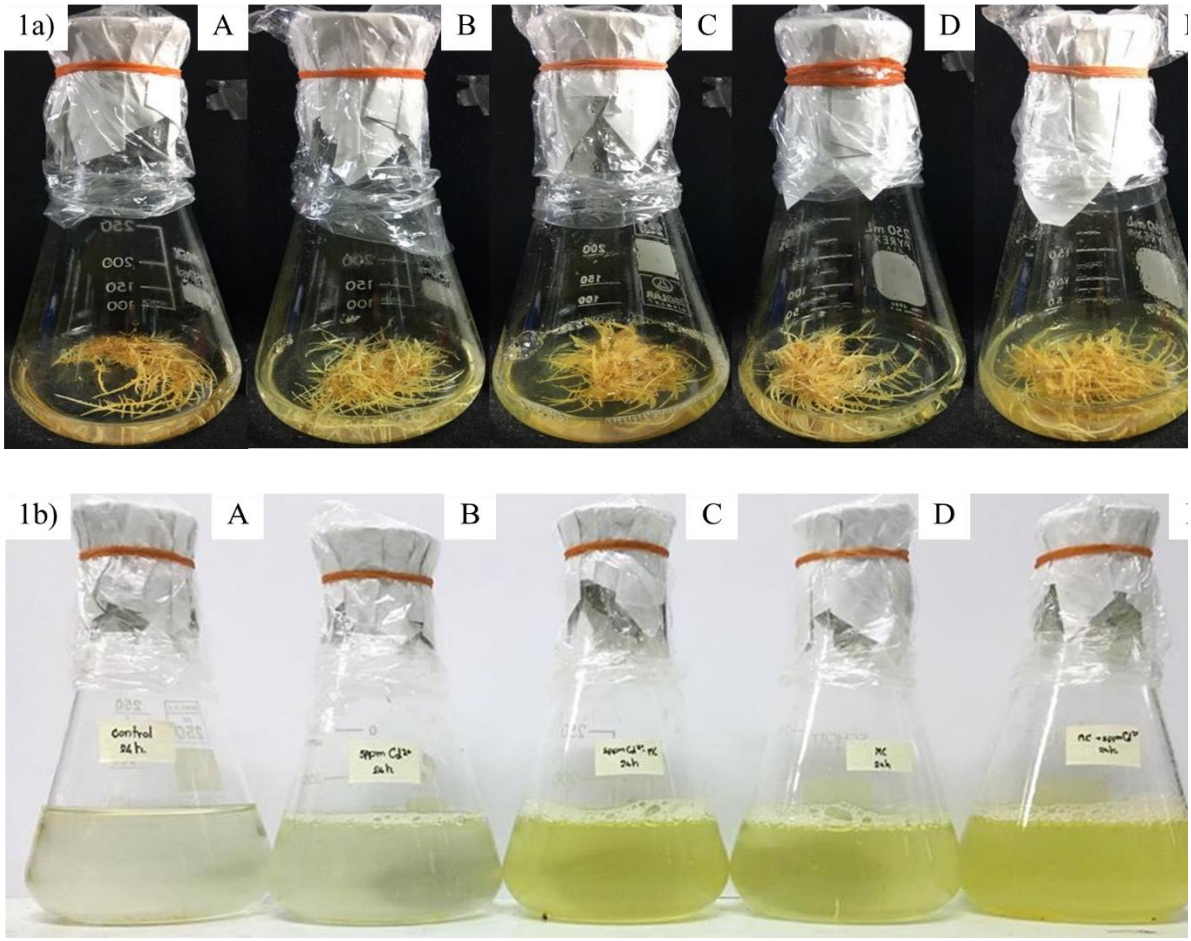

Figure 1. The characteristic of 1a) peanut hairy root tissue and 1b) culture medium of control (A), treatment with Cd (B), Cd followed by MeJA plus CD (C), MeJA plus CD (D), and MeJA plus CD followed by Cd (E).

The optimum concentration of cadmium

The optimum concentration of Cd was chosen by HPLC chromatogram of stilbene compound production (Fig. 2). The results revealed that 5 ppm Cd in combination with MeJA plus CD could induce the highest production of stilbene compounds in hairy root K2-K599 (Fig. 2b). The decrease of stilbene compounds was observed in the higher concentration of Cd; 10 ppm and 50 ppm. This might be due to the effect of high concentration of Cd on root length as described by Minakshi et al.[14]. In addition, high concentration of Cd was reported to induce chromosome bridges, chromosome stickiness and cell alteration in root tips of *Allium cepa var. agrogarum L.*[15]. Thus, the 5 ppm Cd was selected as the optimum concentration to be used for the subsequent experiment.

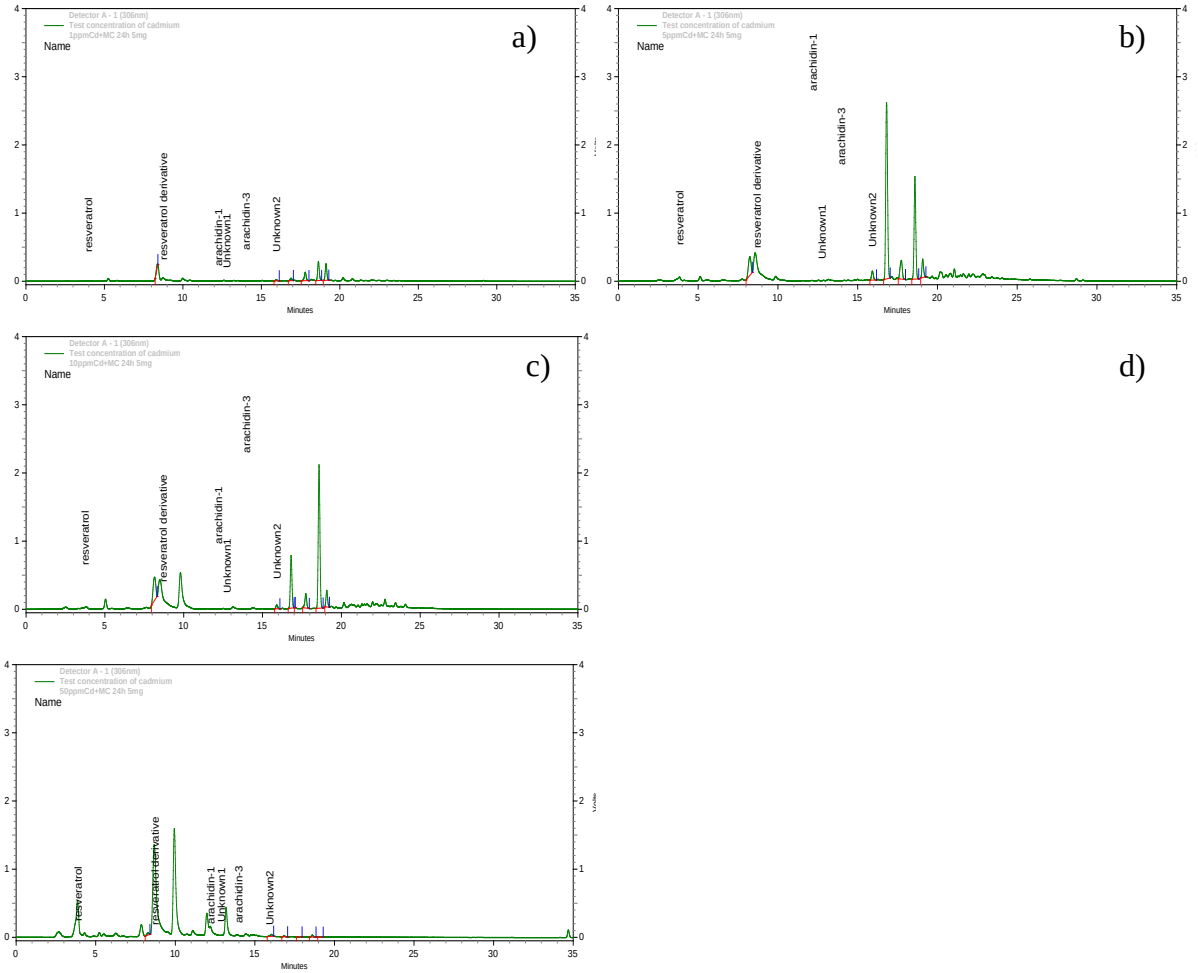

Figure 2. The HPLC chromatograms of the culture medium crude extract treated with a) 1 ppm Cd; b) 5ppm Cd; c) 10 ppm Cd and d) 50 ppm Cd; followed by co-treatment with MeJA plus CD.

The antioxidant assay

The antioxidant activities measured by DPPH and FRAP method of hairy root tissue crude extract (Fig 3a-3b) were lower than the culture medium crude extract (Fig 4a-ab). This was in accordance with the results of Condori et al.[16] which demonstrated the lower level of stilbenoid contents retained in the peanut hairy root tissue compared to the stilbenoid contents detected in medium culture. The antioxidant activity of culture medium crude extract from all treatments except the Cd treated culture revealed the higher antioxidant activity than the control at all-time point (Fig 4a-4b). The co-treatment with MeJA and CD followed by Cd for 48 h exhibited the highest antioxidant activities determined by DPPH and FRAP method with TEAC value of 128.6±1.69 mM Trolox/g DW and AAE value of 462.43±2.76 mg ascorbic acid/g DW, respectively. Zheng et al.[17] also demonstrated that Cd could induce the oxidative stress in plant which caused a high antioxidant compound production. In addition, a positive correlation of the antioxidant capacity measured by DPPH and FRAP assays of both hairy root tissue and culture medium crude extract was determined with r = 0.8490 and r = 0.9877, respectively (Fig 5a-5b). This would be the promising strategy for elicitor treatment to enhance the bioactive antioxidant compounds in hairy root culture.

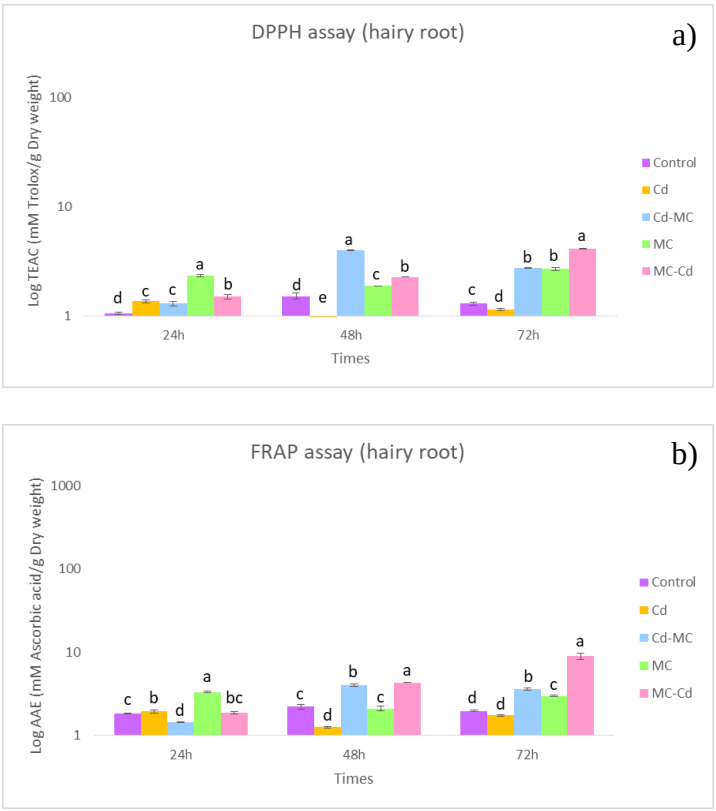

Figure 3. Antioxidant activity of hairy root tissue crude extracts measured by DPPH assay (a) and FRAP assay (b). Data represented as mean ± standard deviation; n=3. Different lowercase letters indicated statistically significant differences (LSD at $P < 0.05$) between treatments for the same time.

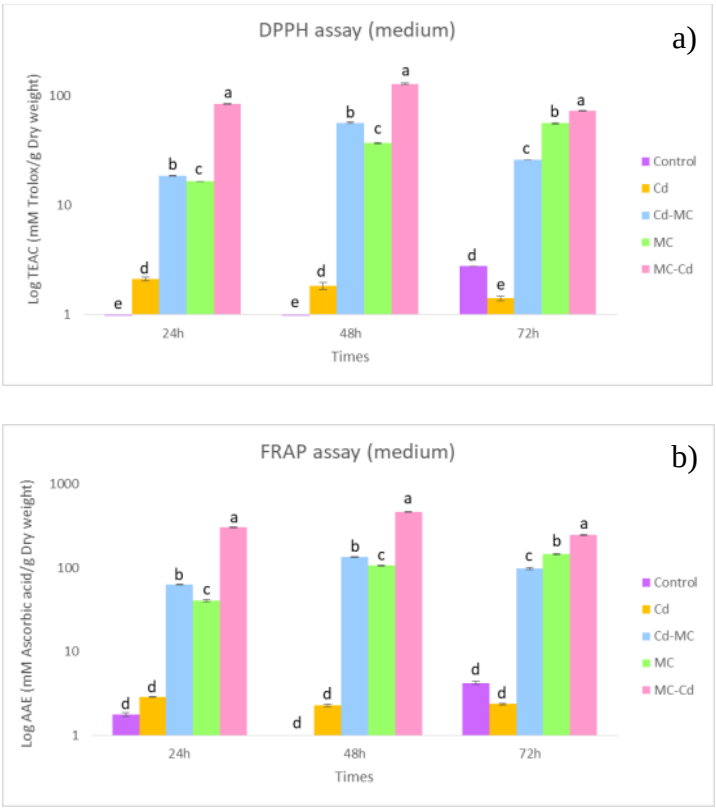

Figure 4. Antioxidant activity of the culture medium crude extracts measured by DPPH assay (a) and FRAP assay (b). Data represented as mean ± standard deviation; n=3. Different lowercase letters indicated statistically significant differences (LSD at $P < 0.05$) between treatments for the same time.

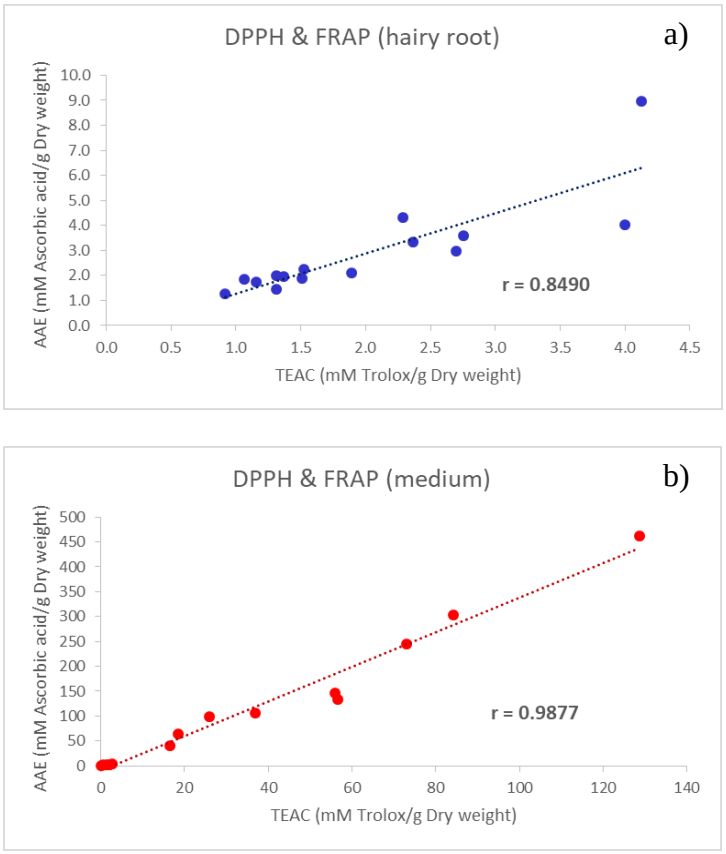

Figure 5. Correlation of the antioxidant capacity measured by DPPH and FRAP assay of hairy root tissue crude extracts (a) and culture medium crude extracts (b).

Conclusion

The antioxidant activity of culture medium crude extract was higher than the hairy root tissue crude extract. The culture medium crude extract of peanut hairy root elicited with co-treatment of MeJA and CD followed by Cd for 48h exhibited the highest antioxidant activity. Even though the high level of antioxidant activity was detected in the medium culture crude extract after elicitation with MeJA and CD followed by Cd, the further experiment should be done to assure that no Cd contamination has been detected in the crude extract.

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Acknowledgements

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