ENHANCEMENT OF BIOACTIVE COMPOUNDS IN *Hylocereus polyrhzus* CALLUS MEDIATED BY PLANT GROWTH REGULATORS AND ELICITORS

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ABSTRACT

Deep red coloured fruit flesh of red pitaya, which is due to betalains pigment, provides an alternative source of antioxidants and natural food colourant. Hence, red pitaya callus culture was established for the production of betalains. Murashige and Skoog (MS) medium supplemented with 2 mg/L 1-naphthaleneacetic acid (NAA) and 4 mg/L thidiazuron (TDZ) was identified as the best treatment to produce pigmented callus with high betalains content. A total of 15 bioactive compounds were identified from the callus compared to only eight bioactive compounds identified from the fresh fruit using LCMS/MS analysis. The callus culture produced seven more bioactive compounds that exist in betacyanins biosynthetic pathway. The study was also carried out to determine the elicitation effect on the established red pitaya callus and betalains biosynthesis in the callus culture using salicylic acid. Four different concentrations of salicylic acid (25, 50, 100 and 200 μM) were added to MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ separately. The morphology of callus produced in all different concentrations of salicylic acid tested was friable and red in colour. Spectrophotometer analysis revealed that 100 μM salicylic acid was determined as optimal concentration to enhance betalains biosynthesis in red pitaya callus culture

Keywords: Bioactive compounds, Betalains, Red pitaya, Callus and Salicylic acid

INTRODUCTION

Red pitaya, *Hylocereus polyrhizus*, is locally known as red dragon fruit. The fruit peel and flesh are deep red in colour when the fruit ripens. It is reported that the peel colour changes from green to deep red while the fruit flesh turns off-white with red into deep red colour upon maturity (Phebe et al. 2009). Pigmentation process involving production of betalains and the intensification of red colour in fruit peel and flesh occurred during maturation. Red pitaya has been reported as rich in betalains and has the similar array of colour pigments found in beetroot (Rebecca et al. 2008, Harivaindaran et al. 2008). Its exotic characteristics with delicious flavour, attractive deep red colour and juicy fruit flesh have made it becomes popular in local fruit market.

Betalains pigment is composed of red-violet betacyanins and yellow betaxanthin. The pigment was reported to have health promoting properties like antioxidant and radical scavenging properties (Strack et al. 2003, Moreno et al. 2008, Han et al. 2009), which can prevent oxidative processes that cause several degenerative diseases in human. On the other hand, betalains pigment with deep red colour is found as a good natural food colourant because of its stability and strong colour, and it does not affect food taste when added to improve food appearance. Hence, deep red coloured fruit flesh red pitaya rich in betalains pigment is identified as a significant source of antioxidants (Rebecca et al. 2010) and an alternative source of natural food colourant (Phebe et al. 2009, Woo et al. 2011a, Woo et al. 2011b, Azeredo 2009).

Production of fruit crop through field cultivation is unable to meet the growing demands of betalains pigment from red pitaya. Growing red pitaya in the field and waiting for it to bear fruits for betalains are laborious and time consuming. In addition, such pitaya plants are susceptible to bacterial soft rot disease

ISSN No 1985-0484

(Masyahit et al. 2009). Therefore, using *in vitro* technology, it is possible to biosynthesise valuable secondary metabolites. Callus culture and plant cell system have become an important strategy for bioprospecting natural products. A callus culture system of red pitaya has been successfully developed to produce betalains pigment (Rogayah et al. 2013, Wee et al. 2013). Using this approach, betalains was continuously synthesised which can be harvested at the callus stage.

It was reported that balanced use of auxin and cytokinin in culture medium significantly increased the growth and production of secondary metabolites in callus system (Reis et al. 2017). Radfar et. al. (2012) reported the use of 2,4-D (1.0 mg/L) and TDZ (2.0 mg/L) as the best combination to induce pigmented callus of *Zaleya decandra* and the pigment accumulated in the callus was confirmed as betalains when subjected to a test using spectrophotometer. In a study of callus initiation and betalains production in *Alternanthera brasiliana*, Reis et al. (2017) stated that a double concentration of cytokinin compared to auxin induced greater accumulation of pigmentation in the callus system.

Different strategies have been used to maximise the yield of secondary metabolites through *in vitro* system including utilisation of elicitors. Generally, elicitors were used to stimulate plant defense and to enhance biosynthesis of secondary metabolites in plant tissue culture. Elicitors give stress effect to activate plant biochemical system, which may increase the biosynthesis of secondary metabolites in plant tissue (Saw et al. 2010). Several types of elicitor such as jasmonic acid, salicylic acid and fungal extract were used to enhance the production in various plant tissue culture systems (Saw et al. 2010, Badrhadad et al. 2013, Mendhulkar & Vakil 2013). In this study, we investigated the effects of different concentrations of plant growth regulators and elicitation effect of salicylic acid for the induction of red pigmented callus and enhancement of betalains content in red pitaya.

MATERIALS AND METHODS

Plant material and establishment of explant

Red pitaya fruits (*Hylocereus polyrhizus*) used in this study were obtained from a local farm located in Sepang, Selangor Darul Ehsan, Malaysia (2°41'28.93"N 101°45'1.9"E). Red pitaya fruits were washed with running tap water and detergent (GLO, Malaysia), followed by surface sterilization using 70% (v/v) ethanol in laminar flow. The fruits were halved and fruit flesh was excised to be used as an explant. All seeds were removed and the 1 cm^3 of the flesh was cultured onto culture medium.

Effect of NAA and TDZ on callus induction and pigment production

Full-strength Murashige and Skoog (Murashige and Skoog 1962) supplemented with 50 mg/L myo-inositol, full-strength MS vitamin, 30 g/L sucrose, and 3 g/L phytagel agar (Sigma, St Louis, USA) was used as control medium throughout the study. In a preliminary study, optimal callus induction was obtained from 2 mg/L NAA treatment (unpublished data). Thus, effects of 2 mg/L NAA in combination with various concentrations of TDZ (1, 2, 3, 4 and 5 mg/L) were investigated. The medium was solidified with 3 g/L phytagel and pH of the medium was adjusted to 5.8 prior to autoclave at 121°C for 15 min.

Effect of elicitors on callus induction and pigment production

MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ was used as basal medium to investigate the effect of elicitors on red pigmented callus induction. Preliminary study was carried out using 100 μM salicylic acid and 100 μM yeast extract as elicitors. Then, elicitation effect of salicylic acid on red pigmented callus induction was further investigated to determine the optimal concentration for enhancement of betalains content in the callus culture. Four different concentrations of salicylic acid at

25, 50, 100 and 200 µM were examined by supplementation into the basal medium separately. Explants cultured on basal medium without salicylic acid was used as a control. The medium was solidified with 3 g/L phytagel and the pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 15 min. Observation was carried out daily for induced callus, callus colour intensity and callus growth stability. Data on callus size (in diameter), morphology (compact, friable or soft and watery) and colour (dark red, red, pale red, yellowish-orange or brown) were recorded every month before subculture.

Culture condition and maintenance of callus culture

All experiments were performed with 20 replications using six explants in each replicate. The cultures were kept in dark condition at 25°C±2°C. The callus cultures produced were subcultured onto fresh medium at 1 month interval.

Analysis of betalains content

One month old callus culture was harvested for pigment content analysis. Betalains content was extracted by grinding 2 g callus in 10 mL distilled water and filtered using 90 mm Whatman filter paper. Water extraction method was used for betalain extraction since betalains is easily soluble in water. The mixture was then pipetted (200 µL per well) into a 96-wells plate and the betalains content was analysed by using a spectrophotometer (Bioteck Quant Microplate Spectrophotometer, Biotek Instrument Inc. Vermont, USA). Betacyanin indicated by red-purple colour pigment was detected at wavelength absorbance of 537 nm. Betalains content (BC) was then determined using the equation BC (mg/L) = [(A x DF x MW x 1000) / (e x 1)], where A is the absorbance readings, DF the dilution factor and 1 is width of the spectrophotometer cell (1 cm). For betacyanin, the extinction coefficient, e is 60 000 L/(mol cm) and MW is 550 g/mol (Stintzing et al. 2003, Ravichandran et al. 2013). Data were analysed in 20 replicates by using Duncan's test in SAS at p-value< 0.05.

Sample preparation for LCMS/MS analysis

Fresh fruit and callus of pitaya were freeze-dried and stored in -20°C prior to extraction. Dried samples (0.5 g) were weighed into 50 mL centrifuge tubes and added with 10 mL of absolute methanol. Samples were homogenised (Ultra Turrax T25, IKE, Germany) for 1 min and vortexed (Multi Reax, Heidolph, Germany) for 15 min. Later, the samples were centrifuged (2-16K Sigma, Germany) at 8900 rpm for 5 min at 4°C. Supernatant was separated from the pellet and the above steps were repeated twice using 10 mL and 20 mL of absolute methanol without going through homogenisation. Approximately 40 mL final volume of extract will be collected for each sample at the end of the extraction. The extract (4 mL) was dried in vacuum concentrator (Concentrator 5301, Eppendorf, Germany) and reconstituted with 30% (v/v) methanol to make a final concentration of 20 mg/mL. The reconstituted extract was filtered via nylon syringe filter (13 mm, 0.45 µm, Agilent) prior to LCMS/MS analysis.

LCMS/MS analysis

A liquid chromatography-mass spectrometry (LCMS/MS) method was developed for the separation and analysis of samples. Mass spectrometer (3200 QTrap, ABSciex, Canada) was coupled with a HPLC unit (1200 series, Agilent Technologies Inc., Waldbronn, Germany) using a diode-array detector (DAD). Mobile phase consisted of buffer A (0.5% formic acid) and B (0.5% formic acid in acetonitrile). The flow rate was set as 0.75 mL/min using C18-gold column (Thermo Scientific, 150 x 4 mm diameter, particle size 5 µm). A gradient setting was developed for the separation which consists of equilibration for 3 min at 95% of buffer A, reduction of buffer A until 70% for 37 min, further reduction of buffer A at 5% for 5

min, instant increment of buffer A within a minute to 95% and equilibration at 95% of buffer A for 4 min. The detection wavelengths were set to 280 and 360 nm at injection volume of 20 µL.

Meanwhile for mass spectrometer, electron-spray ionisation (ESI) in positive mode was chosen for ionisation of metabolites. Temperature was set at 400°C with curtain gas, source gas and exhaust gas were set as 60psi, 100psi and 60psi respectively. Samples were analysed using Analyst software (version 1.4.2).

RESULTS AND DISCUSSION

Red pitaya callus was successfully induced by using single plant growth regulator in a preliminary study. Optimal callus induction was observed in medium supplemented with 2 mg/L NAA (unpublished data). The callus obtained was soft and red in colour. Subsequently, callus induction was carried out to determine the effect of the combination of plant growth regulators on callus induction. Generally, callus induction achieved more than 60% in the combined plant growth regulator treatment. Red pigmented callus with different intensity and morphology was obtained. However, callus induction in medium supplemented with combination of 2 mg/L NAA and 4 mg/L TDZ achieved the highest value of 86.7% among the combination of plant growth regulators tested (Table 1). The callus produced in this treatment was dark red in colour, friable and bigger in size (>2.0 cm). Similarly, Condon (2012) demonstrated that combination of NAA and TDZ produced more callus of *Hylocereus* sp. compared to a single plant growth regulator. On the other hand, Koe et al. (2016) reported that yellow and compact callus of *Hylocereus costaricensis* was induced using the combination of 3.6 ppm 2,4-dichlorophenoxyacetic acid (2,4-D) and 1.8 ppm 6-benzylaminopurine (BAP).

Red pigmented callus of pitaya obtained in the study may be due to the production of betalains in the callus. Callus pigment content analysis was carried out using a spectrophotometer, to detect betalains content in the callus. Callus obtained from 2 mg/L NAA and 4 mg/L TDZ treatment and MS basal salt medium (control) were analysed. Pigment content analysis showed that plant growth regulator enhanced betalains production in red pigmented callus of pitaya. Betalains content obtained from 2 mg/L NAA and 4 mg/L TDZ treated callus was approximately 1.5-fold higher compared to callus cultured on the control medium which was not supplemented with plant growth regulator (Figure 1).

Further analysis using LCMS-MS was also conducted to identify the bioactive compounds present in red pitaya callus produced in MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ. Key marker compound such as betanin (peak 4 in Figure 2a and 2b) was detected 3.5 times higher in callus culture. Besides enhancing the level of betanin, the red pigmented callus obtained by using this treatment was also found to produce other novel bioactive compounds which exist in betacyanins biosynthetic pathway. Red pigmented callus culture produced more bioactive compounds compared to fresh fruit. A total of 15 metabolites were identified in the callus culture while only eight metabolites were identified in red pitaya fresh fruits. The novel bioactive compounds identified included several important betacyanins such as 6'-O-malonyl-2-descarboxy-betanin (peak 7 in Figure 2b), 2' O-apiosyl-phyllocactin (peak 11 in Figure 2b), amaranthin (peak 14 in Figure 2b) and 5,5',6,6'-tetrahydroxy-3,3'-biindolyl (peak 13 in Figure 2b). The results suggested that supplementation of 2 mg/L NAA and 4 mg/L TDZ in callus culture medium may have triggered not only the yield of the above phytochemicals but also modify the metabolic channel of betacyanins biosynthetic pathway in red pitaya callus culture system. Hence, MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ was used as a basal medium for subsequent experiment to investigate the effect of salicylic acid as an elicitor for further enhancement of betalains production.

Table 1. Effects of combination plant growth regulators using 2 mg/L NAA with different concentrations of TDZ on callus induction, size and morphology. Data collected after one month of culture.

Concentrations of TDZ (mg/L)	Callus Induction (%)	Callus size	Callus morphology
0	56.7	+	Soft and watery, pale red
1	61.8	+	Soft and friable, red
2	68.3	++	Soft and friable, red
3	78.3	++	Friable, dark red
4	86.7	+++	Friable, dark red
5	83.3	++	Compact, dark red

\+ < 1.5 cm in diameter
++ 1.5 - 2.0 cm in diameter
+++ > 2.0 cm in diameter

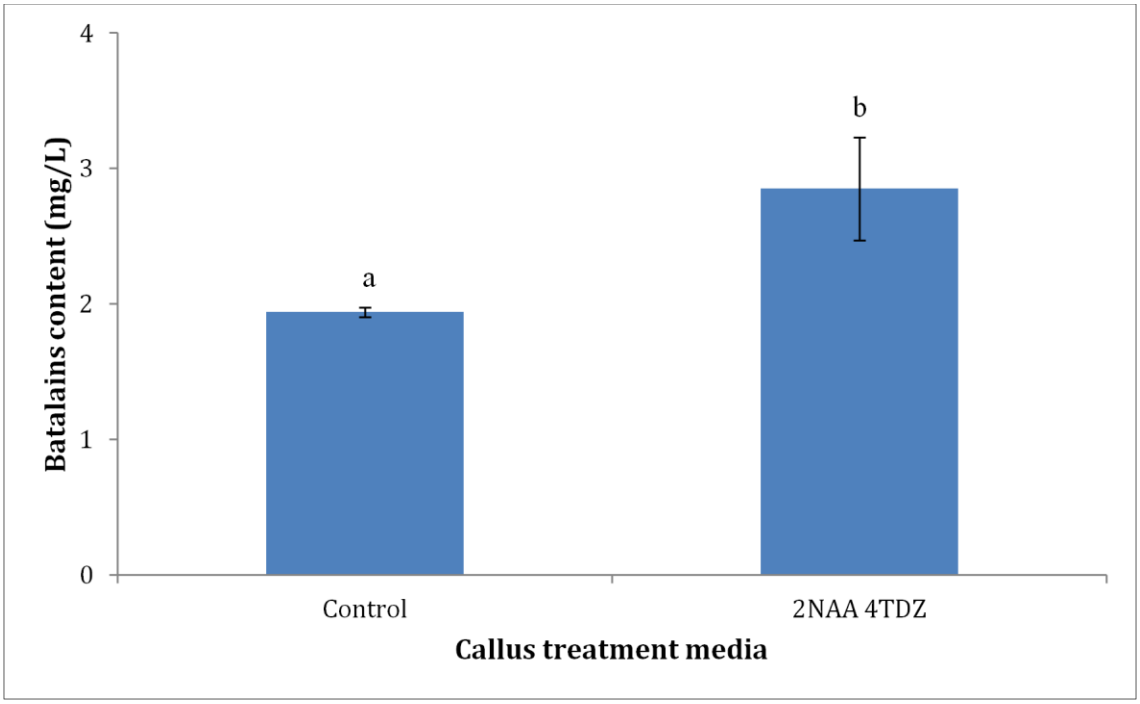

Figure 1. Betalains production in pitaya callus cultured on MS medium as control compared with MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ treated callus culture. Data are presented as mean value ±standard deviation. Means with the same letters are not significantly different at $p<0.05$.

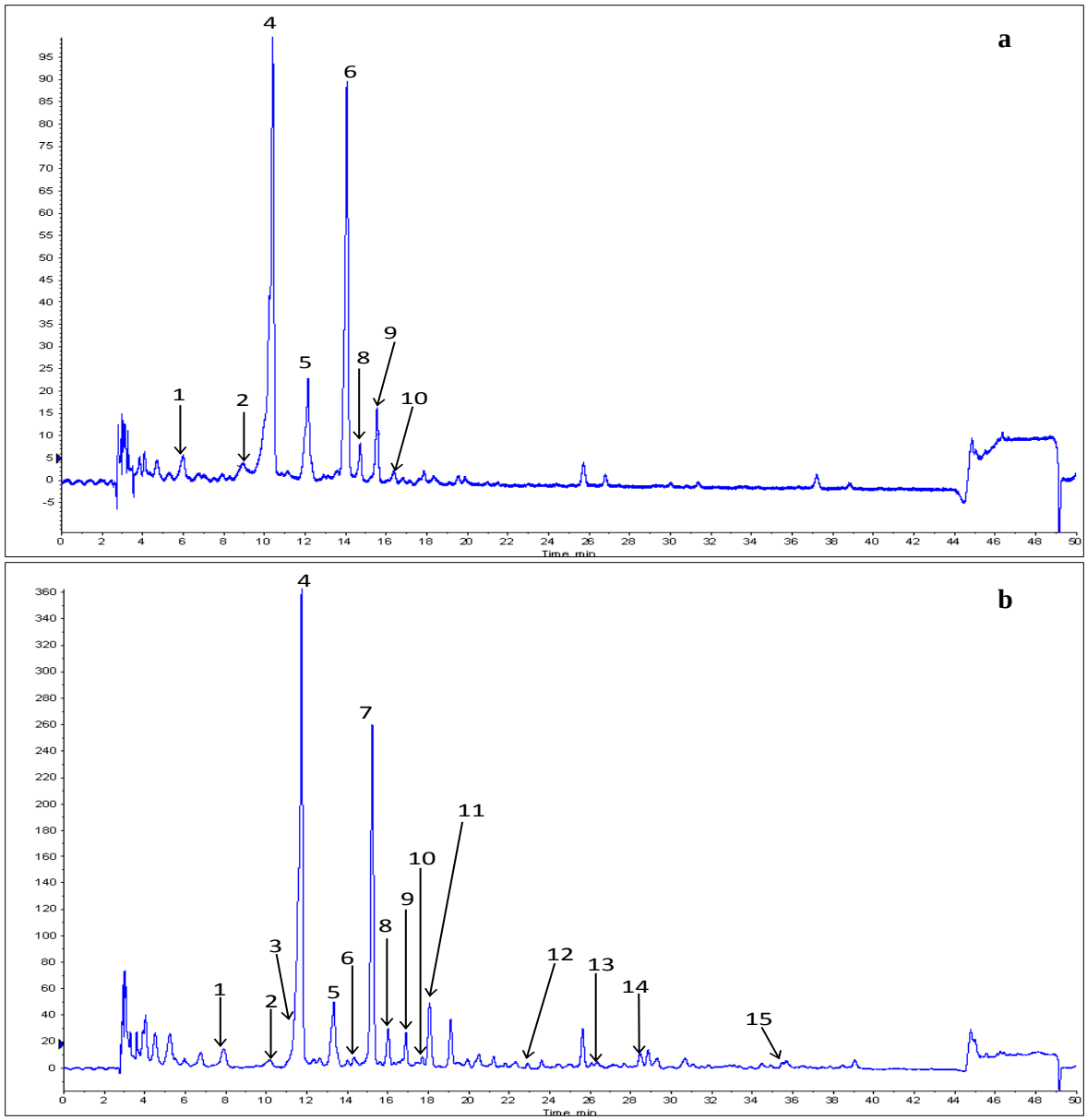

Figure 2. Chromatograms of fresh pitaya (a) and red pitaya callus treated with 2 mg/L NAA and 4 mg/L TDZ (b) obtained from LCMS/MS. Fifteen metabolites identified as Peak 1: 2-descarboxy-cyclo dopa, Peak 2: Indicaxanthin, Peak 3: Isobetanin, Peak 4: Betanin, Peak 5: Isobetanin, Peak 6: Phyllocactin I, Peak 7: 6'-O-malonyl-2-descarboxy-betanin, Peak 8: Isophyllocactin I, Peak 9: 2-descarboxy-betanin, Peak 10: Neo-betanin, decarboxylated, Peak 11: 2'-O-apiosyl-phyllocactin, Peak 12: 6'-O-malonyl-2-descarboxy-betanin, Peak 13: 5,5',6,6'-tetrahydroxy-3,3'-biindolyl, Peak 14: Amaranthin and Peak 15: Decarboxy neobetanidin.

It has been reported in many cases that the addition of appropriate elicitors can significantly increase the yield of plant secondary metabolites (Zhao et al. 2005). Preliminary study had been carried out to investigate elicitation effects of two different elicitors, salicylic acid and yeast extract on red pitaya callus induction and betalains production in the culture. Friable and red pigmented callus (Figure 3a) was obtained in culture medium supplemented with 100 μM salicylic acid while medium containing 100 μM yeast extract produced soft, yellow-orange colour and stunted growth callus (Figure 3b). Pigment content analysis also showed lower betalains was produced in yeast extract treated callus compared to salicylic acid treated callus (data not shown). Further investigation was conducted focusing on determining optimal level of salicylic acid for enhancing the callus growth and betalains production.

Salicylic acid was found to affect pitaya callus colour, texture and growth when added in different concentrations. In general, red pigmented callus in different intensity was produced in medium supplemented with different concentrations of salicylic acid tested; only small amount of callus was observed showing yellow-orange colour. Up to 91% of callus obtained in medium containing 25 μM salicylic acid was deep red in colour, compared to 89%, 86% and 74% at 50, 100 and 200 μM salicylic acid culture media. Friable callus was formed in medium added with salicylic acid while soft callus was observed in medium without salicylic acid. All the callus generated with 25 and 50 μM salicylic acid supplemented media increased growth twice higher compared to callus cultured in 100 and 200 μM supplemented media.

Subsequently, effect of salicylic acid on betalains production in red pitaya callus culture was examined. Salicylic acid was reported to increase synthesis of anthocyanin in grape cell cultures (Saw et al. 2010), alpha-tocopherol in *Elaeagnus angustifolia* cell suspension culture (Badrhadad et al. 2013) and flavonoids in *Andrographis paniculata* cell cultures (Mendhulkar & Vakil 2013). In this study, the investigation revealed that betalains biosynthesis in red pitaya callus culture was improved by salicylic acid addition. Based on the absorbance reading at 537 nm, betalains content in callus produced in 25 and 50 μM salicylic acid was not significantly different (p-value<0.05) from callus cultured in MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ (Figure 4). On the other hand, betalains content obtained from callus cultured in 100 μM salicylic acid supplemented medium achieved the highest betalains yield with 2-fold increase among various concentrations of salicylic acid tested. However, an increased concentration of salicylic acid to 200 μM did not further enhance betalains production of the callus. The betalains content in 200 μM salicylic acid-treated callus was lower than 100 μM salicylic acid-treated callus and did not differ significantly from callus cultured on the control medium.

Salicylic acid (400 μM) was reported to increase betalains content in *Alternanthera tenella* leaves by converting tyrosine into betacyanins (Rodrigues-Brandao et al. 2014). In this study, 100 μM salicylic acid was determined as optimal concentration to enhance betalains biosynthesis in red pitaya callus culture. Even though 25 and 50 μM salicylic acid treated callus showed higher growth but betalains content was significantly 2-times higher in 100 μM salicylic acid treated callus.

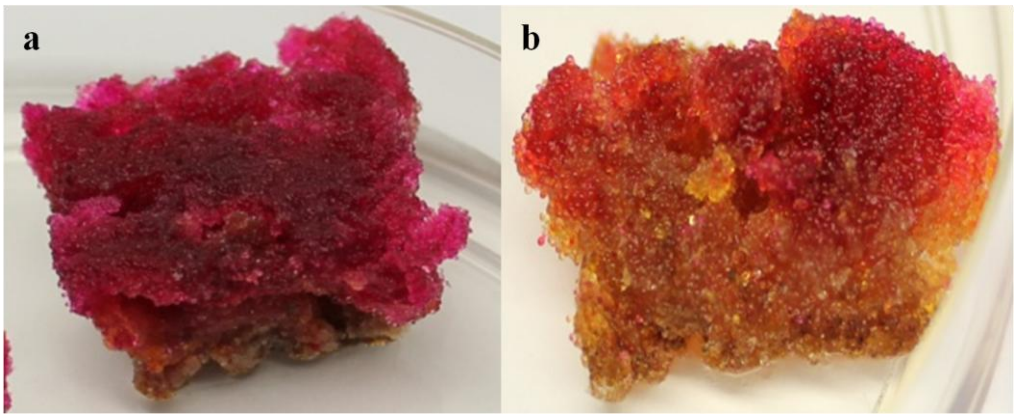

Figure 3. Red pitaya callus obtained from MS medium supplemented with 2 mg/L NAA and 4 mg/L TDZ and elicited with (a) 100 µM salicylic acid and (b) 100 µM yeast.

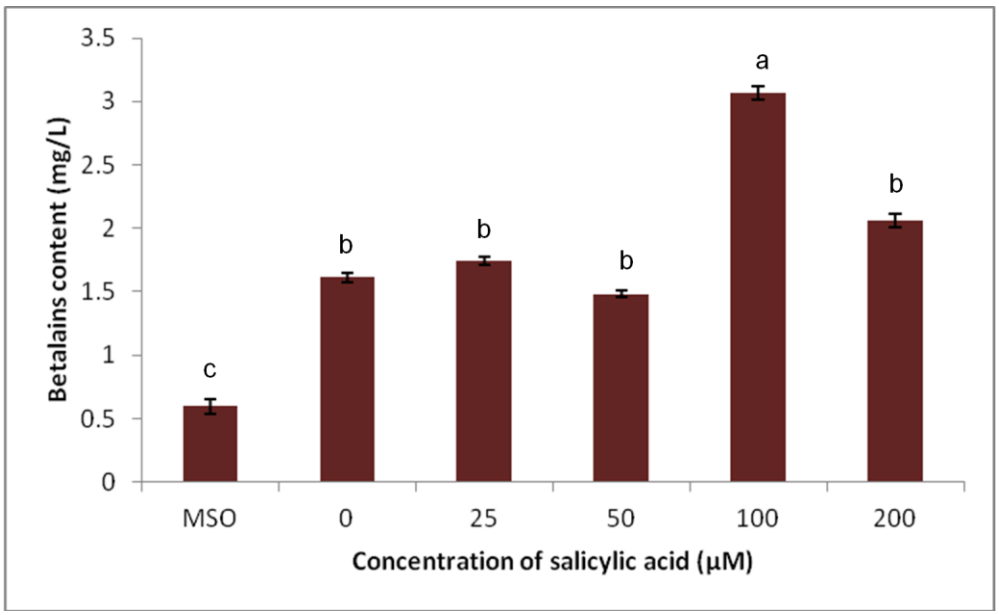

Figure 4. Effect of salicylic acid on betalains biosynthesis in red pitaya callus culture at different concentrations (0, 25, 50, 100 and 200 µM) compared to MS medium without hormone and salicylic acid (MSO). Data are presented as mean value ± standard deviation. Means with the same letters are not significantly different at $p<0.05$.

CONCLUSION

Medium supplemented with 2 mg/L 1-Naphthaleneacetic acid (NAA) and 4 mg/L thidiazuron (TDZ) was identified as the best condition for production of dark red and friable pigmented callus with high betalains content. Detection of seven more bioactive compounds were successfully identified from red pitaya callus using LCMS/MS analysis and these bioactive compounds were only found in red pitaya callus culture and not in red pitaya fresh fruit. The present study also demonstrated that different concentrations of salicylic

acid influenced red pitaya callus growth, colour intensity and betalains production in the culture. Based on the results obtained, 100 μM salicylic acid was found to be the most favourable in betalains production in callus culture among the concentrations investigated.

ACKNOWLEDGEMENTS

This work was supported by the Malaysian Agricultural Research and Development Institute (MARDI) Grant 2100300270001.

REFERENCES

Azeredo HMC, 2009. Betalains: properties, sources, applications and stability – a review. *Int J Food Sci Technol* 44, 2365-2376.

Badrhadad A, Piri K and Ghiasvand T, 2013. Increasing of alpha-tocopherol in cell suspension cultures *Elaeagnus angustifolia* L. *Int J Agric Crop Sci* 5(12), 1328-1331.

Condon WP, 2012. Clonal Propagation of Pitaya (*Hylocereus* sp.) Hybrids. Masters of Science Thesis. University Of Florida USA.

Han XH, Gao ZJ and Xiao XG, 2009. Enzymes and genes involved in the betalain biosynthesis in higher plants. *Afr J Biotechnol* 8(24), 6735-6744.

Harivaindaran KV, Rebecca OPS and Chandran S, 2008. Study of optimal temperature, pH and stability of dragon fruit (*Hylocereus polyrhizus*) peel for use as potential natural colorant. *PakJ Biol Sci* 11, 2259-2263.

Koe WWS, Jeevandran Sundarasekar J, Sathasivam K and Subramaniam S, 2016. Effects Of Plant Growth Regulators On Seed Germination And Callus Induction Of *Hylocereus costaricensis*. *Pak J Bot* 48(3), 977-982,

Masyahit M, Sijam K, Awang Y and Ghazali M, 2009. First report on bacterial soft rot disease on dragon fruit (*Hylocereus* spp.) caused by *Enterobacter cloacae* in peninsular Malaysia. *Int J Agric Biol* 11, 659–666.

Mendhulkar VD and Vakil MMA, 2013. Elicitation of flavonoids by salicylic acid and *Penicillium expansum* in *Andrographis paniculata* (Burm. f.) Nees. cell culture. *Res Biotechnol* 4(2), 01-09.

Moreno DA, García-Viguera C, Gil JI and Gil-Izquierdo A, 2008. Betalains in the era of global agri- food science, technology and nutritional health. *Phytochem Rev* 7, 261-280.

Murashige T and Skoog F, 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Plant Physiol* 15, 473-497.

Phebe D, Chew MK, Suraini AA, Lai OM and Janna OA, 2009. Red-fleshed pitaya (*Hylocereus polyrhizus*) fruit colour and betacyanin content depend on maturity. *Int Food Res J* 16, 233-242.

Radfar M, Sudarshana MS and Niranjan MH, 2012. Betalains from stem callus cultures of *Zaleya decandra* L. N. Burm. f. - A medicinal herb. *J Med Plants Res* 6(12), 2443-2447.

Ravichandran K, Saw NMMT, Mohdaly AAA, Gabr AMM, Kastell A, Riedel H, Cai Z, Knorr D and Smetanska I, 2013. Impact of processing of red beet on betalain content and antioxidant activity. *Food Res Int* 50, 670-675.

Rebecca OPS, Boyce AN and Chandran S, 2010. Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*). *Afr J Biotechnol* 9(10), 1450-1454.

Rebecca OPS, Zuliana R, Boyce AN and Chandran S, 2008. Determining pigment extraction efficiency and pigment stability of dragon fruit (*Hylocereus polyrhizus*). *J Biol Sci* 8, 1174-1180.

Reis A, Kleinowski AM, Klein FRS, de Souza RTT, do Amarante L and Braga EJB, 2017. Callus induction and betacyanin quantification by HPLC/MS-MS in *Alternanthera brasiliana* (L.) Kuntze. *Hoehnea* 44(1): 90-95.

Rodrigues-Brandao I, Kleinowski AM, Einhardt AM, Lima MC, do Amarante L, Peters JA and Braga EJB, 2014. Salicylic acid on antioxidant activity and betacyan in production from leaves of *Alternanthera tenella*. Ciencia Rural, Santa Maria 44, 1893-1898.

Rogayah S, Wee CY, Siti Ratnadia J and Indu Bala J, 2013. Enhancement of betalain production in dragon fruit using callus culture. *Abstract of 24th Malaysian Society of Plant Physiology Conference 2013*, Prinz Park Resort, Terengganu, Malaysia.

Saw NMMT, Riedel H, Kutuk O, Ravichandran K and Smetanska I, 2010. Effect of elicitors and precursors on the synthesis of anthocyanin in grape *Vitis vinifera* cell cultures. *Energy Res J* 1(2), 189-192.

Stintzing FC, Schieber A and Carle R, 2003. Evaluation of colour properties and chemical quality parameters of cactus juices. *Eur Food Res Technol* 216, 303-311.

Strack D, Vogt T and Schliemann W, 2003. Recent advances in betalain research. *Phytochemistry* 62, 247-269.

Wee CY, Nor Hidayu CA, Siti Fatiha Y, Rogayah S and Indu Bala J, (2013). Evaluation of sucrose concentration and gelling agent on pitaya callus morphology and betalains production in the culture. *Abstract of 24th Malaysian Society of Plant Physiology Conference 2013*, Prinz Park Resort, Terengganu, Malaysia.

Woo KK, Agou LS, Soong WK and Tang PY, 2011a. Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). *Amer J Food Tech* 6(2), 140-148.

Woo KK, Agou LS, Soong WK and Tang PY, 2011b. Stability of the spray-dried pigment of red dragon fruit [*Hylocereus polyrhizus* (Weber) Britton and Rose] as a function of organic acid additives and storage conditions. *Philippine Agric Scientist* 94(3), 264-269.

Zhao J, Davis LC and Verpoorte R, 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol Adv* 23(4), 283-333.