AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Comparative flower pigment study of orchid plants

[1]Suat Hian Tan, [1]Shahida Abdul Manap, [1]Md. Rezaul Karim, [1]Shah Samiur Rashid, [2]Maziah Mahmood, [3]Nyuk Ling Ma

[1]Industrial Biotechnology Programme, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, LebuhrayaTun Razak, 26300, Gambang , Kuantan, Pahang, Malaysia.
[2]Department of Biochemistry, Faculty of Biotechnology and Biomoleculer Sciences, Universiti Putra Malaysia, 43300, Serdang, Malaysia.
[3]School of Fundamental Science, Universiti Malaysia Terengganu, Mengabang Telipot, 21030 Kuala Terengganu, Terengganu, Malaysia.

ARTICLE INFO

Article history:
Received 30 September 2014
Received in revised form
17 November 2014
Accepted 25 November 2014
Available online 13 December 2014

Keywords:
Chlorophy, Carateroid, Anthocyanin, Phenylalanine ammonia-lyase

ABSTRACT

Background: *Orchidaceae* species has developed into one of the significant industrial products in agricultural industry globally. Beside as potted flower, they serve as cut flowers since they have eye-catching pigments in the flower petals. **Objective:** The main pigments from orchid's flower petals were investigated and their relations with phenylalanine ammonia-lyase (PAL) activity were evaluated. **Results:** Total anthocyanin content of six different orchids' petals was determined specthrophotometrically and the value ranged from 0 mg/g (in *Dendrobium* Shavin white) to 2.128 mg/g (in *Mokara* Aranda). Total anthocyanin content was found to be the highest when compare to β-carotene and chlorophyll content. In correlation analysis, PAL activity was found to be significant positive correlated with the anthocyanin content. **Conclusion:** The results indicate the potential for PAL enzyme as a biomarker for flower colour in orchids.

To Cite This Article: Suat Hian Tan, Shahida Abdul Manap, Md. Rezaul Karim, Shah Samiur Rashid, Maziah Mahmood, Nyuk Ling Ma., Comparative flower pigment study of orchid plants. ***Adv. Environ. Biol., 8(20),*** *20-24, 2014*

INTRODUCTION

Orchids belong to the largest family of flowering plants in the world [1]. According to Sugapriya [2], more than 25,000 to 30,000 species indicating 600-800 genera of orchids have been recorded. These species are classified as *Oncidium, Cattleya, Epidendrum, Cymbidium, Phalaenopsis, Vanda, Dendrobium, Phaphiopedilum, Brassica, Laelia, Mokaram Aranchnis and Miltonia*. Production of Orchid has turn into the major floral crop and its financial value has been increasing notably year by year. There is a huge commercial potential of orchids in domestic and global market [3] and colour of the flowers have immense commercial implications.

Relationship of flower colour and pigment compositions has been established by many researchers [4]. Other than the aesthetic aspects of flower color, floral pigments have important role for pollination of flowers by animals. During the development of flowers in nature the pigments can be modified/changed in many ways such as, at emergence and post-pollination [5, 6]. There are ranges of biochemical mechanisms involved in the change of colour both with in flowers and in isolated pigments. Some of the factors influencing colour aretemperature, co-pigments, pH, metals, sugars, anthocyanin stacking and cell shape [7, 8].

Plant pigmentation is involved three major pigments which included anthocyanin, carotenoid and chlorophyll. Chlorophyll is usually accountable for the green colour. Anthocyanin is in red and blue while carotenoids are responsible for yellow [9]. Gorgeous and eye-catching colours of flowers are mainly result from anthocyanin, which are developed in the petals. Anthocyanin is synthesized throughout phenylpropanoid pathway which is mainly catalyzed by PAL [10]. To the best of our knowledge, the study on the association of PAL and anthocyanin synthesis within the orchid's plants was relatively low. Therefore, in this study, the major flower pigments extracted from the petals of orchids and their relations with (PAL) activity were evaluated. The finding of such study will have significant on the application of PAL for the evaluation of pigmentation in petals.

Corresponding Author: Suat Hian Tan, Industrial Biotechnology Programme, Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang, LebuhrayaTun Razak, 26300, Gambang , Kuantan, Pahang, Malaysia.
Phone: +6095492397, Email: tshian@ump.edu.my; onefist82@gmail.com,

MATERIALS AND METHODS

Materials:

All chemicals used were of analytical reagent grade except for trans-cinnamic acid, cyanidin and L-phenylalanine were high-performance liquid chromatography grade which purchased from Sigma. Methanol, acetone, β-carotene, Boric acid, ethanol were purchased from Chemolab. HCl, petroleum ether, $CaCO_3$ were purchased from Fluka Chemicals and chloroform, tris, KCl, mercaptoethanol were purchased from Merck.

Plant Materials:

Six different species of orchids were bought from Central Market Kuantan, Pahang, which are *Mokara* Pink, *Mokara* Aranda, *Mokara* Gold Nugget, *Ascocenda* Dong Tarn, *Dendrobium* Sonia 17 and *Dendrobium* Shavin White. They were maintained in greenhouse, Universiti Malaysia Pahang. Fully bloom flowers of each orchid was collected for further analysis.

Total Anthocyanin Content:

Total anthocyanin content was determined with a modified method from Bharti and Khurana [11]. Half of the known fresh flower weight (g) was immersed in 10ml methanol with 1%HCl for 24h in the dark. Then, Back extraction was used to portion the anthocyanin from chlorophyll pigment with 10ml chloroform and 9ml distilled water. Two hundred µl of the sample was measured at 530nm and 657nm by using Infinite® 200 PRO series microplate readers. The difference in absorbance 530nm and 657nm were recorded.

Total β-carotene Content:

Total β-carotene content was determined according to Norhazira et al [12] with slight modification. A known fresh weight (g) was ground in the mixture of 5ml of cold acetone and 5ml light petroleum with mortar and pestle. It was filter with Whatman No 1 filter paper then centrifuge at 5000rpm for 5min under 4˚C. The supernatant was taken and the absorbance value was read at 415nm. The supernatant was taken and the absorbance value was read at 415nm.

Total Chlorophyll Content:

Total chlorophyll content was determined according to Tan [13]. A known fresh weight (g) was ground using mortar and pestle in 10ml 80% (v/v) acetone until all the colour was released from the tissues. Whatman No 1 filter paper was used to filter the extract. The excess sample was washed with acetone until the sample was colourless. The extract and washing acetone were made up to 20ml. The extract was measured at 645nm and 663nm. Chlorophyll a and chlorophyll b content was calculated from the following formulae:

$$\text{Chlorophyll a}(\text{mg/g}) = \frac{12.3\,A_{663} - 0.86\,A_{645}}{\alpha \times 1000 \times W} \times V$$

$$\text{Chlorophyll b}\,(\text{mg/g}) = \frac{19.3\,A_{645} - 3.6\,A_{663}}{\alpha \times 1000 \times W} \times V$$

Total chlorophyll = chlorophyll a +chlorophyll b

Where A_{645} and A_{663} are the absorbance at 645nm and 663nm, V is the volume in ml, α is the length of the light path in the cell (1cm) and W is the fresh weight in grams.

Preparation of Crude Enzyme:

A known fresh weight (g) was ground using mortar and pestle in 10ml Tris-HCl of pH 7.0 and 7.5 and 10ml of borate buffer was used for pH 8.0, 8.5, 9.0and 9.5 in a precooled mortar and pestle. The homogenate was centrifuged at 4°C for 20min at 5000rpm. The supernatant was used for PAL assay and soluble protein content determination.

PAL Assay:

PAL enzyme activity was determined with a modified method of El-Shora [14]. One ml reaction mixture containing 0.45ml 100mM Tris-HCl pH 8.5 with 1mM 2-mercaptoethanol, 0.5ml 50mM L-phenylalanine and 0.05ml of the crude enzyme was incubated at 30°C for 15min. After 15min, the mixture was terminated by the addition of 0.5ml 6M HCl. Reaction mixture without crude extract was used as a blank and reaction mixture with zero time reaction was used as control. The procedure was repeated by using crude enzyme with different pH. The sample was measured at 290nm.

RESULTS AND DISCUSSION

The absorbance spectra of flower pigments can be considered as its fingerprint and it is a simple way to identify and quantify pigments present in a mixture [15] especially for flower pigment. In this study it was estimated the total anthocyanin, β-carotene, and chlorophyll content in flower petals of the orchids: *M.* Pink, *M.* Aranda, *M.* Gold Nugget, *A.* Dong Tarn, *D* Sonia 17, *D* Shavin White (Table 1). The amounts of pigment (anthocyanin, β- carotene and chlorophyll) within the flower petals of various orchid hybrids were ranging from 0 and 2.13 mg/g, 0.004 and 0.10 mg/ g and 0.01 and 0.07 mg/g, respectively .

Anthocyanin was found to be the major pigments in *M.* Pink, *M.* Aranda, *M.* Gold Nugget, *A.* Dong Tarn, *D* Sonia 17. This is due to the morphological and colour characteristic of the flower as they accumulate anthocyanin content in their petals as compared to *D.* Shavin White which is white in colour. The differential of anthocyanin content among the orchids may due to the genetic factor in each orchid, which regulates the transcriptional production through enzyme action [16]. It was also observed by Mizuta *et al* that flowers with white petals are devoid of anthosyanin and anthocyanin constitution of the purple group flowers is more varied than that of the red group flowers, and this wider variety among purple flowers contributes to extending the diversity of flower color [17].

Second major pigment detected was β-carotene as shown in table 1. β-carotene was found be highest in *M.* Gold Nugget (0.11 mg/g) and the least amount was found in *D* Shavin White (0.004 mg/g). Usually, coloured flowers contains higher β-carotene content and it was evident from the study by Tatsuzawa *et al* [18] Matsui and Nakamura [19] that combination of anthocyanins and carotenoids might control the different varieties of petal colors in the range of orange, red, pink, purple etc.

Chlorophyll pigment was detected in all orchids studied which are range from 0.01 to 0.07 mg/g. Interestingly, it was found that when the flower petal contains lower anthocyanin content, the total chlorophyll content exhibited a higher value relatively. It is not necessary that the green part of the plants only need to have chlorophyll but the petals also have photosynthetically active chloroplasts even if they are pink or some other color than green. The flower is maximally photosynthetic until just before it opens, then it slows. The other pigments that color the petals just mask the chlorophyll [20].

Table 1: Total anthocyanin, β-carotene, and chlorophyll content in flower petals of orchids.

Flower petals of orchids	Total anthocyanin content (mg/g)	Total β-carotene content (mg/g)	Total chlorophyll content (mg/g)
M. Pink	0.44	0.08	0.05
M. Aranda	2.13	0.06	0.02
M. Gold Nugget	1.30	0.11	0.04
A. Dong Tarn	0.89	0.01	0.01
D Sonia 17	0.07	0.01	0.06
D Shavin White	0	0.004	0.07

Generally, enzyme activity tent to increase with the increase of pH value. Figure 4 shows the effect of different pH on PAL enzyme activity of orchid flower. The result indicated that PAL enzyme activity increases as the pH increases from7.0 to 8.5 due to increase stability of the active site. However, beyond pH 8.5, the PAL enzyme activity was drop drastically. This is because pH 9.0 no longer can support the active site of the PAL enzyme as it started to be denatured or less stable.

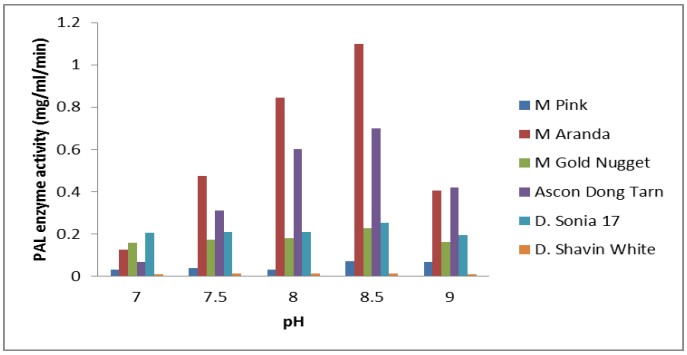

Fig. 4: Effect of different pH on PAL activity of orchid flowers.

It is observed that anthocyanin content correlated well with the PAL enzyme activity at pH 8.5 with R^2= 0.6698 (Figure 5). Higher PAL activity is associated with higher anthocyanin content in colour petals orchids. These results therefore suggest that the change in PAL activity was implicated in the change in anthocyanin aggregation during the maturity of colour flower petals. According to Wang et. al. [21], changes in anthocyanin aggregation can occur separately besides changes in PAL activity; the increase of anthocyanin may also implicate other enzymes among leucocyanidin and cyanidin glycosides.

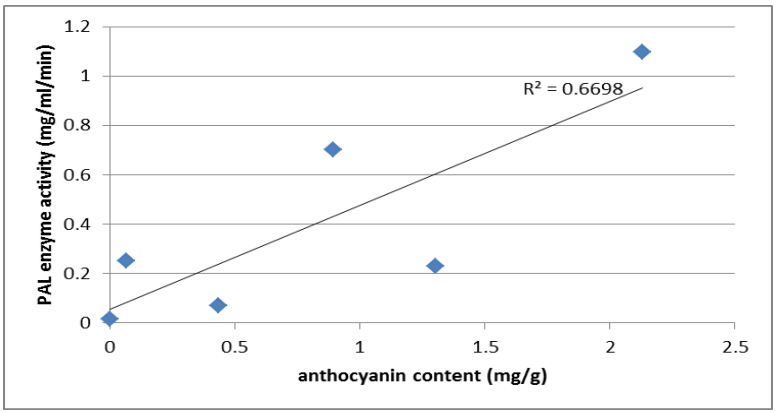

Fig. 5: Correlation between PAL activities with anthocyanin content of orchid flowers.

Conclusion:

In this study, the flower pigments in the petals of orchids and the PAL activity has been investigated. The result indicated that the petals which have intense colour have high amount of anthocyanin content whereas for those are pale in colour have high amount of chlorophyll content. PAL activity was shown to be positively correlated with the total anthocyanin content in the orchid flower petals. The result of this study suggests that PAL enzyme may contribute as a biomarker for flower colour in orchids.

ACKNOWLEDGEMENT

The authors are grateful for the financial support through the research grant RDU 120502 and RDU 130609. We would like to thank all FIST staff, Universiti Malaysia Pahang for their fully cooperation.

REFERENCES

[1] Orchidaceae, 2014. Retrieved from http://en.wikipedia.org/wiki/Orchidaceae.
[2] Sugapriya, S., 2009. Evaluationof Dendrobium Orchids under Greenhouse Condition. Degree Thesis. University of Agriculture Sciences, Dharwad.
[3] FAMA, 2014. Federal Agricultural Marketing Authority: Ministry of Agriculture and Agro-Based Industry Malaysia. Retrieved from http://www.fama.gov.my/en/home#.VGBzePmUcb0.
[4] Kishimoto, S., K. Sumitomo, M. Yagi, M. Nakayama, A. Ohmiya, 2007. Three routes to orange petal color via carotenoids components in 9 Compositae species. Journal of Japanese Soc for Horticultural Science, 76: 250-7.
[5] Rebelo, A.G., W.R. Siegfried, 1984. Colour and size of flowers in relation to pollination of Erica species. Oecologia, 65: 584-901.
[6] Cannon, J., M. Cannon, 1994. Dye plants and dyeing. The Herbert Press; 1994 [in association with Kew].
[7] Shoji, K., N. Miki, N. Nakajima, K. Momonoi, C. Kato, K. Yoshida, 2007. Perianth bottom-specific blue color development in tulip cv. M urasakizuisho requires ferric ions. Plant & Cell Physiology, 48: 243-51.
[8] Bowles, D., E-K. Lim, B. Poppenberger, F.E. Vaistij, 2006. Glycossyltransferases of lipophilic small molecules. Annual Review of Plant Biology, 57: 567-97.
[9] Merzlyak, M.N., A.E. Solovchenko and A.A. Gitelson, 2003. Reflectance Spectral Features and Non-destructive Estimation of Chlorophyll, Carotenoid and Anthocyanin Content in Apple Fruit. Postharvest Biologyand Technolgy, 27: 197-211.
[10] Vagas, F.D., A.R. Jimenez and O. Paredes-Lopez, 2000. Natural Pigments: Carotenoids, Anthocyanins and Betalains- Characteristics, Biosynthesis, Processing and Stability. Critical Reviews in Food Science and Nutrition, 40: 173-289.

[11] Bharti, A.K. and P.J. Khurana, 2003. Molecular Characterization Of Transparent Testa (tt) Mutants of Arabidopsis thanliana (Ecotype Estland) Impaired in Flavonoid Biosynthetic Pathway. Plant Science, 165: 1321-1332.
[12] Norhaiza, M., M. Maziah and M. Hakiman., 2009. Antioxidative Properties Of Leaf Extracts of a Popular Malaysian Herb, Labisia pumila. Journal of Medicinal Plants Research, 3: 217-223.
[13] Tan, S.H, 2010. Production of Flavonoids in Pegaga (Centella asiatica L. Urban) Cell Suspension Culture. Doctorate Thesis. University Putra Malaysia, Malaysia.
[14] El-Shora, H.M., 2002. Properties of Phenylalanine Ammonia-Lyase from Marrow Cotyledons. Plant Science, 162: 1-7.
[15] Schoefs, B., 2004. Determination of Pigments in Vegetables. Journal of Chromatography A, 1054: 217-226.
[16] Schluter, P.M. and F.P. Schiesti, 2008. Molecular Mechanisms of Floral Mimicry in Orchids. Trends in Plant Science, 13: 228-235.
[17] Mizuta, D., B. Takuya, M. Ikuo, N. Akira, K. Nobuo, 2009. Co mparison of flower color wit h anthocya nin composition patterns in ever green azalea. Scientia Horticulturae, 122: 594-602.
[18] Tatsuzawa, F., K. Ichihara, K. Shinoda, K. Miyoshi, 2010. Flower colours and pigments in Disa hybrid (Orchidaceae). South African Journal of Botany, 76: 49-53.
[19] Matsui, S., 1992. Varietal differences in flower colours of Cattleya aurantiaca. Research Bulletin of the Faculty of Agriculture, Gifu University, 57: 181-185.
[20] Griesbach, R.J., 2005. Biochemistry and Genetics of Flower Color. Plant Breeding Reviews, pp: 89-99.
[21] Wang, H., O. Arakawa and Y. Motomura, 2000. Influence Of Maturity And Bagging on the Relationship Between Anthocyanin Accumulation and Phenylalanine Ammonia-Lyase (PAL) Activity in 'Jonathan' Apples. Postharvest Biology and Technology, 19: 123-128.