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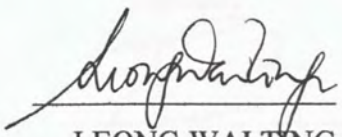
The materials in this thesis are original except for quotations, except summaries and references, which have been duly acknowledged.

12 March 2007



LEONG WAI TING

HS2004-3491

BORANG PENGESAHAN STATUS TESIS@

JUDUL: ANTHOCYANIN ANALYSIS OF ~~KA~~ VANDA MERICLONE USING HPLC

Ijazah: Sarjana Muda ** Sains dengan kepujian (HG07 Bioteknolog

SESI PENGAJIAN: 2004/2005

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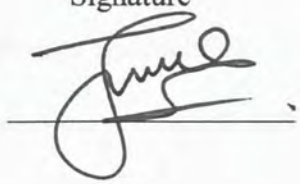

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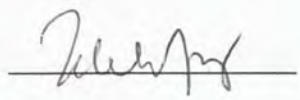

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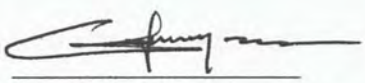

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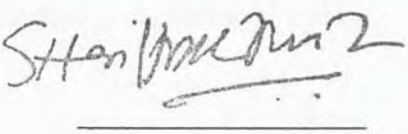

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ABSTRAK

Objektif kajian ini adalah untuk membuat analisis tentang kuantiti and kualiti pigmen antosianin dan menganalisiskan hubungan antara kuantiti bagi enam jenis antosianin yang tidak sama dengan kebolehan bunga sp. *Vanda* untuk mengeluarkan kewangian. Pada masa yang sama, variasi elit klon anak pokok untuk pembiakan telah dicari daripada kajian ini. Setiap anak pokok dikultur pada keadaan persekitaraan yang sama. Untuk proses pengekstrakan pigmen, 0.1% asid hydroklorik dalam methanol digunakan untuk mengekstrak antosianin. Selepas proses pengekstrakkan, "high performance liquid chromatography" (HPLC) digunakan untuk mengenalpasti and mengukur kandungan bagi enam jenis antosianin utama iaitu delphinidin, sianidin, petunidin, pelargonidin, peonidin dan malvidin. Puncak (peaks) di dalam kromatogram bagi setiap sampel dikenalpasti mengikuti 'absorption spectra' dan masa kesinggahan (retention time) berbanding dengan piawai. Kawasan di bawah puncak bagi puncak yang dipilih digunakan untuk mengira kandungan jenis antosianin yang didapati dalam anak pokok sp. *Vanda*. Keputusan ini menunjukkan delphinidin tidak terdapat dalam anak pokok sp. *Vanda* dan sianidin pula hanya didapati di beberapa sample spesis *Vanda*. Sample ke-36 mengandungi malvidin dan peonidin yang tertinggi antara semua anak pokok *Vanda dearei* manakala sample ke-35 mempunyai kandungan pelargonidin yang tertinggi. Sample ke-22 mengandungi petunidin yang tertinggi. Antara anak pokok *Vanda helvola*, sample ke-25 mempunyai kandungan petunidin dan pelargonidin yang paling tinggi berbanding yang lain manakala sample ke-7 mengandungi peonidin yang paling tinggi. Sample ke-28 pula mengandungi malvidin yang terbanyak. Kesimpulannya, ini membuktikan jenis-jenis antosianin dapat dianalisis melalui HPLC dan jenis antosianin yang dikenalpasti dalam kajian ini dapat mewakili warna pada bunga pokok dewasa di Taman pertanian Lagud Seberang, Tenom. Begitu juga, anak pokok yang mengandungi malvidin mungkin dapat mempengaruhi penghasilan bunga yang wangi. Kajian yang dijalankan adalah penting untuk kenalpasti variasi anak pokok berdasarkan penghasilan bunga yang wangi kelak.

ABSTRACT

The objective of this research was to discover the quantity and quality of anthocyanin and analyzing the correlation between the quantity of types of anthocyanins and the ability of producing floral fragrant by *Vanda* sp. The seedlings were grown under same condition; 24 hours exposed to light with 25 ± 2^0C. In the extraction process, 0.1% (v/v) hydrochloric acid in methanol acid solution used to extract anthocyanins. After the extracting process, high performance liquid chromatography was used to identify and measure the amount of six main types of anthocyanins; delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin. The peaks of the chromatograms for each sample were identified by comparing the absorption spectra and retention to that of authentic standards. The area under the curve of the wanted peaks used to find out the amount of the particular anthocyanins in the *Vanda* sp. seedlings. The results shown that delphinidin was not found in the *Vanda* sp. seedlings. The sample No.36 of *Vanda dearei* had the highest content of malvidin and peonidin, while sample No.35 of *Vanda dearei* had the highest amounts of pelargonidin and No.22 was found to contain the highest amount of petunidin. Besides, among 50 seedlings of *Vanda helvola*, the samples No. 25 produced the highest amount of petunidin and pelargonidin. While sample No.7 had the highest content of peonidin and No.28 has the highest content of malvidin. In conclusion, it was proven that the types of anthocyanins produced by *Vanda* sp. was suitable to be analyzed by using HPLC and the particular types of anthocyanins gave the colours to the flowers of the adult plant. Meanwhile, the seedlings composed of high level of malvidin may have the potential in producing fragrance during blooming in adult. The result of this research will be very beneficiary to expand on further study on variations plants in order to observe the relationship between floral fragrance and the amount of malvidin produced.

TABLE OF CONTENT

LIST OF TABLE

LIST OF FIGURES

LIST OF PHOTOS

LIST OF SYMBOLS, UNITS AND ABBREVIATION

B = boron
Cl = chlorine
Co = cobalt
Cu = copper
I = iodine
Mo = molybdate
Zn = zink
Mn = manganese
nm = nanometer
kJ = kilojoule
UV = ultraviolet
m^2s^{-1} = meter cube per second
1 nm = 10^{-9}m
Avogadro No. = $6.023x10^{23}$
CS = chalcone synthase
CI = chalcone isomerase
DHK = dihydro-kaempferol
F3H = flavanone 3-hydroxylase
DHQ = dihydroquercetin
DHM = dihydromyricetin
DFR = dihydroflavonol 4-reductase
FS = flavonol synthase
ANS = anthocyanidin synthase
3GT = anthocyanin glucosyltransferase
DCMU = 1-dimethyl urea
NA = non-acclimated
BAMT = benzoic asid methyl tranferase
MT = methyltransferase

SAM = S-adenosyl-L-methionine
SAMT = salicylic acid methyl transferase
OMT = *O*-methyltransferase
LDOX = leucoanthocyanidin dioxygenase
HPLC = high performance liquid chromatography
TFA = trifluoroacetic acid
DAD = photodiode array detecton
FeNa EDTA = iron sodium ethylenediaminetetraacetic acid
PAL = phenylalanine ammonia lyase

LIST OF APPENDIX

CHAPTER 1

INTRRODUCTION

The *Vanda* came from a Sanskrit word referring to *Vanda roxburghii* R.Br. of India, the type of the genus, which is now known as *V. tessellate* (Roxb.) D.Don (Chan *et al*., 1994). The *Vanda* brings the meaning of the flower with its scents, colour and shape. Most of the orchids are epiphyte, but sometimes they are found in the lithophytic (grows on bare rock or stone) or terrestrial. There are 3 categorizes of *Vanda*: i.e., strap-leaved, semi-terete and terete. The orchids in strap-leaved group have wider leaves, while orchids in terete group have round elongated leaves look like pencil, and for the orchids in semi-terete group have the leaves with the characters of both previous groups. Among these three groups, the terete group needs a high intensity of light for a better growth. There are around 80 species of *Vanda* be found. They are mostly distributed in China, Himalaya mountains, Indonesia, North Australia, South East Asia (include Malaysia, Indonesia and Philipine), Papua New Guinea and East China Sea (Hodgson *et al*., 1991).

In this experiment, *Vanda helvola* and *Vanda dearei* species were being examined. In Borneo, *Vanda helvola* can be found in Mount Kinabalu and Tambunan area while *Vanda dearei* can be found in East and West of Kalimantan (around Sekayan River area, Kutai), Sabah (Kinabatangan District, Sapulot area, Tenom District and Tomani area) and Sarawak (Kuching area). *Vanda helvola* was suitable to grow in the mixed hill-forest, riverine forest, mixed lower montane forest and

generally on solids derived from sandstones, shales and mudstones while *Vanda dearei* suitable to grow in the riverine forest. However, the deforesting of the origin habitat of these species for country development made them facing extinction. The seedlings took long period for growth and inflorescence. Basically, they would not able to grow if there were competition and threat from animals and other plant species. The specific name of *Vanda helvola* came from Latin language 'helvus' referred to the pinkish colour of the flower. The form collected at Kundasang on the slopes of Mt. Kinabalu has beautiful cinnamon brown to coppery red coloured sepals and petals, similar to forms found in Sumatra (Chan *et al.*, 1994). The *Vanda dearei* is the species of great horticultural interest to breeders before and after the war. Its large, very fragrant flowers have been used in several well-known early hybrids including *Vanda* Tan Chay Yan, produced in Singapore.

In this project, study of anthocyanin had been on *Vanda helvola* and *Vanda dearei.* The seedlings derived from seeds of these two species had been cultured for 1 year and 5 months, and the pods were taken from Taman Pertanian Lagud Seberang Tenom, Sabah. The purpose of this research was to study and analyzing anthocyanin in *Vanda sp.* orchids which were cultured in the condition of 24 hours light at 25 ± 2^{0}C. Any elite found among the seedlings would be chose for reproducing young plant for the purpose of conservation. However, in this research, the original characteristics of species would be preserved because the unique of the original characteristics of the species could not be affected and altered. The genetic components of the seedlings in this research were the same with the genetic components of the wild type of the parent plants in natural environment. In this research, the seedlings were exposed to one condition only which all seedlings were under the same intensity of light for 24 hours.

Anthocyanin is the photoreceptor compound involved in this research. The quality and quantity of the anthocyanin had been studied and analyzed for the correlation to the quality of the flowers of *Vanda helvola* and *Vanda dearei*. This was then followed by indicating the correlation between the biosynthesis of anthocyanins with the biosynthesis of other fragrant compounds from *Vanda helvola* and *Vanda dearei*. There were six common anthocyanidins found in higher plants which are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Cooper-Driver, 2001). These six anthocyanidins were being examinated from the seedlings of *Vanda helvola* and *Vanda dearei* in this research. The types and the quantity of the types of anthocyanins had to be found out and that type of anthocyanin might be a potential indicator that influencing the releasing of the fragrance compounds of the flowers. For an example, the higher the amount of malvidin extracted from seedlings of *Vanda helvola* or *Vanda dearei*, the more fragrant compound (i.e., iso-eugenol) would be expected to be produced by the flower during influorescent. This indication was refered to the most recent research on *Petunia hybrida* cultivars that the volatile compounds released by day from the flowers 40 commercial *Petunia hybrida* cultivars were analyzed. The three cultivars with solid deep-blue flowers that accumulate malvidin in corollas with high tissue pH were found to emit abundant iso-eugenol as the principal floral fragrance. Therefore, association between the floral fragrance and the other floral traits such as floral anthocyanin composition and corolla-tissue pH was discussed (Nakamura *et al.*, 2006).

Different anthocyanins give different colours of petals (i.e, brick-red pelargonidin, red cyanidin and blue delphinidin glucosides) (Cooper-Driver, 2001).

The types and amount of the anthocyanins accumulate in *Vanda helvola* and *Vanda dearei* could be compared through this research because the colour pattern of the *Vanda helvola* flower was different with the flower colour of *Vanda dearei*, this indicated that the types of anthocyanins accumulate in these two species might be different.

High performance liquid chromatography (HPLC) was encouraged to use for anthocyanins analysis in this research. This was because HPLC is commonly used to identify main types of anthocyanins (qualitative and quantitative) in many researches. For an example, major anthocyanidins (Cyanidin, Peonidin and Malvidin) were determined by HPLC after extraction and hydrolysis with hydrochloric acid (Nakamura *et al*., 2006).

The objectives of this research were:

1) Quantity and quality analysis of anthocyanin and comparison between *Vanda helvola* and *Vanda dearei*.
2) The potential of anthocyanin in producing floral fragrance during influorecent.

Therefore, in this research, much of experiences of works in laboratory and knowledge about anthocyanin pigment been obtained.

CHAPTER 2

LITERATURE REVIEW

2.1 *Vanda helvola*

Vanda is a monopodial orchid (derived from Greek, *mono*-single, and *pado*-a feet). The meaning of monopodial is having a single shoot and one root where the plants keep on growing and reproduction. Sometimes, they are litophytic/terrestrial which grown on the stones and on the soil. *Vanda helvola* are mostly epiphytic herb with thick robust roots. The stems elongating to over 100cm, erect but more commonly semi-pendulous to pendulous, lower portion of stem partly or wholly covered by old leaf-sheaths, older stems having one or more new growths superposed on the basal part, forming a small cluster of plants. The leaves size are around 15-20 x 3.3-3.5cm, close together, 2-3 cm apart, bases concealing stem, curving, rigid, leathery, apex obtusely unequally bi-lobed and toothed. The flowers inflorescence in the kind of 'lateral inflorescence' with 8-11cm long, usually 1 or 2 produced at time at regular intervals during the year, laxly 4- to 6- flowered; floral bracts 3-4 mm long, broadly triangular. The diameter of the flowers are 4-5 cm across, wide opening, fleshy, waxy, resupinate, lasting up to one week, scented and the colour is very variable. The unique of the *Vanda* is on the flower's characteristics. *Vanda* is a mericlone which bring the meaning of vegetative plants. When any part of the plant is cut and growing, the young plant produced will be the same with the mother plant. Some of the *vanda* species are hybrid to produce

variation (Hodgson *et al.*, 1991). The colours of sepals are from dull pale yellow-brown to coppery red with dull yellow edges. The dorsal sepals on the upper part of the flowers are smaller than the lateral sepals. The size of dorsal sepal is around 1.3-1.5 x 1.2 cm, while the lateral sepal's size is around 1.6-1.8 x 1.3-1.5 cm. The petals are small with only 1.3-1.5 x 0.8-1.1 cm recurved at base. The lip is tri-lobed, attached to a short column-foot, slightly hinged and movable. The size of fruit produced is 1.3 cm long. The characteristic of *Vanda helvola* is the flowers are produced at regular intervals throughout the year, the coppery red mountain form being apparently more strongly scented. The form collected at Kundasang on the slopes of Mt. Kinabalu had beautiful cinnamon brown to coppery red coloured sepals, and petals, similar to forms found in Sumatra. Paler yellowish to pinkish forms occur elsewhere in the Crocker Range (Chan *et al.*, 1994). The sample of *Vanda helvola* in this experiment was taken from Taman Pertanian Lagud Seberang Tenom.

The colour of *Vanda helvola* flowers shown in photos 2.1 and 2.2 are in a bit reddish and a bit yellowish.

Photo 2.1 Adult plant of *Vanda helvola* during inflorescence.

Photo 2.2 *Vanda helvola* flower in short distance.

2.2 *Vanda dearei*

Types of *Vanda dearei* from Borneo are distributed in East and West of Kalimantan, Sabah and Sarawak. *Vanda dearei* is also an epiphytic herb with thick, robust roots. The stems are up to 100 cm or more long, erect to semi-pendulous, stout, the older ones having one or more new growths superposed on the basal leafless part, eventually forming a cluster of new plants, almost wholly enclosed in leaf sheaths. When they inflorescence, usually 1 or 2 produced at a time at regular intervals during the year, up to 15 cm long, laxly 4- to 6-flowered; floral bracts 3-5 cm long. The diameter of the flowers is 7-9 cm across, fleshy, strongly scented, remaining open for one week. The sepals and petals overlapping, in most plants becoming strongly reflexed after the first 2 days, pale cream, yellow (particularly in Sarawak populations) or pale yellow, flushed salmon or dull brown towards the tips and edges, rarely yellow suffused with brown all over. The lip is with white side lobes, mid-lobe white at base, with red lines at the junction with the side lobes, dark yellow at apex. The size of leaves is around 35-45 x

3-3.8 cm, crowded, lingulate, apex bi-lobed and irregularly toothed, thick, coriaceous. The dorsal sepals are similar shape with lateral sepals. Size of Dorsal sepals is around 3.5-4 x 2-3 cm, while the size of lateral sepals is around 3-4 x 2-3 cm. Their lip is tri-lobed. This species was of great horticultural interest to breeders before and after the war. Its large, very fragrant flowers have been used in several well-known early hybrids including *Vanda* Tan Chay Yan, produced in Singapore. Cultivated plants produce flowers frequently throughout the year (Chan *et al.*, 1994). The sample of *Vanda dearei* in this experiment is taken from Taman Pertanian Lagud Seberang Tenom.

Photo 2.3 Adult plants of *Vanda dearei* during inflorescent.

Photo 2.4 *Vanda dearei* flower in short distance.

The classification is shown below (Hodgson *et al.*, 1991):

Alam	: Plantae
Phylum	: Magnoliophyte
Class	: Liliopsite
Order	: Asparagales
Family	: Epidendroideae
Tribe	: Vandeae
Subtribe	: Sarcanthinae
Alliance	: Vanda
Genus	: *Vanda*

Other species of vanda are:

- *Vanda dearei* (Malaysia).
- *Vanda helvola* (Malaysia - Borneo).
- *Vanda brunnea* (China – Yunnan to Indo-China).
- *Vanda caerulea : Blue Orchid* (Assam to China – S. Yunnan).
- *Vanda alpina* (Himalaya to China – S.Yunnan).
- *Vanda arbuthnotiana* (India).
- *Vandal celebica* (Indonesia – Sulawesi).
- *Vandal chlorosantha* (Bhutan).
- *Vandal concolor* (S. China to Vietnam).

REFERENCE

Anderson, O.M., 1992. Anthocyanins from reproductive structures in Pinaceae. Biochem. Syst. and Ecol. 20, 145-148.

Ando, T., Takahashi, M., Nakajima, T., Toya, Y., Watanabe, H., Kokubun, H., Tatsuzawa, F., 2004. Delphinidin accumulation is associated with abnormal flower development in petunias. Phytochemistry **65**, 2219–2227.

Brouillard, R., Wigand, M.C., Dangles, O., Cheminat, A., 1991. PH and solvent e.ects on the copigmentation reaction of malvin with polyphenols, purine and pyrimidine-derivatives. J. Chem. Soc., Perkin Trans. **2** (8), 1235-1241.

C.L Chan, A.Lamb, P.S Shim & J.J Wood, 1994, *Orchids of Borne Vol.1:Introduction and A Selection of sp.*, Sabah Society and the Royal Botanic Garden, Kew, Kota Kinabalu, Sabah.

Courtney-Gutterson, N., Napoli, C., Lemieux, C., Morgan, A., Firoozabady, E., Robinson, K., 1994. Modification of flower color in florists Chrysanthemum-production of a white- flowering variety through molecular-genetics. Biotechnology 12 (3), 268-271.

Creasy, L.L. 1968a. Phytochemistry. 7:1743.

Creasy, L.L. 1968b. Proc. Amer. Soc. Hort. Sci. 93:716.

Creasy, L.L. 1974. phytochemistry 13:1391.

Dudareva N., Pichersky E. 2000, Biochemistry of plant volatile. *Plant Physiology*. **122**, 627-633.

Dudareva N., Pichersky E. & Gershenzon J., 2004, Biochemistry of Plant Volatile. *Plant Physiology*. **135**, 1893-1902.

Downs, R.J., Siegelman, H.W., Butler, W.L. and Hendricks, S.B. 1965. Nature 205: 909.

Drum-Herrel, H., 1984, Blue/UV light effects on anthocyanin synthesis. In: Senger, H. (Ed.), Blue light effects in biological systems. Springer-Verlag, Berlin, pp. 375-383.

Edgerton, L.J. & Blanpied, G.D. 1968. *Nature* 29:1064.

Faust, M, 1965. ph.D. thesis. Physiology of Anthocyanin in Developmetn in McIntosh Apples. Cornell university. U.S.A.

Field T.S., Lee D.W. & Holbrook N.M., 2001, Why Leaves Turn Red in Autumm. The Role of Anthocyanins in Senescing Leaves of Red-Osier Dogwood. *Plant Physiology*, **127**, 566-574.

Figueiredo, P., Elhabari, M., Toki, K., Saito, N., Dangles, O., Brouillard, R., 1996. New aspects of anthocyanin complexation. Intramolecular co-pigmentation as a means for colour loss? Phytochemistry **41** (1), 301-308.

Franga S.C., Roberto P.G., Marins M.A., puga R.D., Rodrigues A. & Pereira J.O., 2001, Biosynthesis of secondary metabolites in sugarcane. *Genetics and Molecular Biology*, **24**, 1-4.

Gershenzon J., McConkey M.E. & Croteau R.B., 2000, Regulation of Monoterpene Accumulation in Leaves of Peppermint. *Plant Physiology*, **122**, 205-213.

Gillian A. Cooper-Driver., 2001, Contributions of Je.rey Harborne and co-workers to the study of anthocyanins. *Phytochemistry*. **56**, 229-236.

Goto, T., Tamura, H., Kawai, T., Hoshino, T., Harada, N., Kondo, T., 1986. Chemistry of metalloanthocyanins. Annals of the New York Academy of Sciences **471**, 155-173.

Gould K.S., Markham K.R., Smith R.H. & Goris J.J., 2000, Functional role of anthocyanins in the leaves of *Quintinia serrata* A.Cunn., *Journal of Experimantal Botany*, **51**, 1107-1115.

Harborne, J.B., Cli.ord, H.T., 1967. Anthocyanin composition of and distribution in the Poaceae (Gramineae). Proceedings of the Linnean Society of London 178, 125.

Harborne, J.B., 1976. A unique pattern of anthocyanins in Daucus Carota and other Umbelliferae. Biochem. Syst. and Ecol. 4, 111-115

Harborne, J.B., Stirton, J.Z., 1980. Two distinctive anthocyanin patterns in the Commelinaceae. Biochem. Syst. and Ecol. 8, 285-287.

Harborne, J.B., Williams, C.A., Mayo, S.J., 1981. Anthocyanin pigment and leaf flavonoids in the family Araceae. *Phytochemistry,* **20**, 217-234.

Harborne, J.B., 1992b. Flavonoid pigments. In: Rosenthal, G.A., Berenbaum, M.R. (Eds.), Herbivores: Their Interactions with Secondary Plant Metabolites, Vol. 1, The chemical participants. Academic Press, New York, pp. 389-427.

Harborne, J.B., Boulter, D., Turner, B.L. (Eds.). 1970. Chemotaxonomy of the Leguminosae. Academic Press, London, p. 612.

Harborne, J.B., 1996. Chemotaxonomy of anthocyanins and phytoalexins in the Compositae. In: Hind, D.J.N., Beentje, H.J. (Eds.), Proceedings of the International Compositae Conference, Kew, 1994, Royal Botanic Gardens, Kew, vol. 1, pp. 207-218

Harborne, J.B., Williams, C.A., Eagles, J., 1989b. Leaf flavonoid diversity in the Australian genus Patersonia. *Phytochemistry,* **28**, 1891-1896.

Harborne, J.B., Williams, C.A., 1998. Anthocyanins and other flavonoids. Natural Products Reports 15, 631-652.

Harborne, J.B., Williams, C.A., 1995. Anthocyanins and other flavonoids. Natural Products Reports 12, 639-657.

Harborne, J.B., 1965c. Flavonoid pigments. In Bonner, J., Varner, R. (Eds), Plant Biochemistry, 2nd Edition. Academic press, London, pp. 618-640.

Harborne, J.B., Boardley, M., 1985. The widespread occurrence in nature of anthocyanins as zwitterions. Zeitschrift fuÈ r Naturforschung 40, 305-308.

Harborne, J.B., Williams, C.A., 1998. Anthocyanins and other Flavonoids. Natural Products Reports **15**, 631-652.

Harborne, J.B., 1993. Biochemistry of plant pollination. Introduction to Ecological Biochemistry, 4th Edition. Academic Press, London, pp. 36-70.

Haslam, E. 1993. Nature's palette. Chemistry in Britain, October, 875-878.

Haslam, E., 1995. Fruit and floral pigmentation. Rev. Prog. Coloration **25**, 18-28.

Hodgson, M., Paine, R. & Anderson. N, 1991, *Letts Guide to Orchids of the World*, Charles Letts & Co Ltd, United Kingdom.

Holton, T.A., Cornish, E.C., 1995. Genetics and biochemistry of anthocyanin biosynthesis. The Plant Cell 7, 1071-1083.

Irani N.G. & Grotewold E., 2005, Light-induced Morphological Alteration in Anthocyanin-Accumulating vacuoles of maize cells, *BMC Plant Biology*, **5**, 1-15.

Jurgens A., Witt T. & Gottsberger G., 2002, Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). *Biochemical Systematics and Ecology,***30**, 383-397.

Knoll, H.A., Brady, N.C. & Lathwell. D.J. 1964. *Soil Sci Soc Amer. Proc.* 28:400.

Kolosova, N., Gorenstein, N., Kish, C.M., Dudareva, N., 2001. Regulation of circadian methyl benzoate emission in diurnally and nocturnally emitted plants. The Plant Cell **13**, 2333–2347.

Lee H.S., 1992, Chromatography analysis of anthocyanins. Journal of Chromatography, **624**, 221-234.

Longo L. & Vasapollo G., 2006, Extraction and identification of anthocyanins from *Smilax aspera* L. berries. *Food Chemistry*, **94**, 226-231.

Markham, K.R., 1990. Bryophyte flavonoids, their structures, distribution and evolutionary significance. Proc. Phytochem. Soc. Eur. 29, 143-159.

Muhammad M., Muhammad J., Jalal-ud-din B. & Rehman K.K., 2004, Impact of light intensity on flowering time and plant quality of *Antirrhinum majus* L. Cultivar Chimes White. *Jornal of Zhejiang University Science*, **5**, 400-405.

Mol, J., Grotewold, E., Koes, R., 1998. How genes paint flowers and seeds. *Trends in Plant Sciences,* **3**, 212-217.

Mori K., Sugaya S. & Gemma H., 2005, Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Scientia Horticulturae*, **105**, 319-330.

Nakamura K., Matsubara K., Watanabe H., Kokubun H., Ueda Y., Oyama-Okubo N., Nakayamam M.& Ando T., 2006, Identification of Petunia hybrida cultivars that diurnally emit floral fragrances. Sci*entia Horticulturae*. **108**, 61-65.

Nakatsuka T., Nishibara M., Mishiba K. & Yamamura S., 2005, Temporal expression of flavonoid biosynthesis-related genes regulates flower pigmentation in gentian plants. *Plant Science*, **168**, 1309-1318.

Oren-Shamir M. & Levi-Nissim A., 1997, UV-light effect on the leaf pigmentation of *Cotinus coggygria* 'Royal Purple'. *Scientia Horticulturae*, **71**, 59-66.

Orryllagha J.C. & Grotewold E., 2004, The biology and structural distribution of surface flavonoids. *Plant Science*, **2**, 1-19.

Pastenes C., Santa-Maria E., Infante R. & Franck N., 2003, Domestication of the Chilean guava (*Ugni molinae*), a forest understorey shrub, must consider light intensity, *Scientia Horticulturae*, **98**, 71-84.

Pecket, R.C. & Bassim, T.A.H. 1974. Phytochemistry 13:1359.

Porter A.M., 1937, Effect of Light Intensity on the Photosynthetic Efficiency of Tomato Plants. *Plant Physiology*, **12**, 225-253.

Premkumar A., Mercado J.A. & Qucsada M.A., 2001, Effects of in-vitro culture conditions and acclimation on the contents of Rubisco, leaf soluble proteins, photosynthetic pigments, and C/N ratio, *Journal of Plant Physiology*, **158**, 835-840.

Rhodes, M.J.C. & Wooltorton, L.S.C. 1977. phytochemistry 16:655.

Sadik, S. & Ozbun, J.L. 1967. Can J. Bot 45:955.

Saito, N., Ku, M., Tatsuzawa, F., Lu, T.S., Yokoi, M., Shigihara, A., Honda, T., 1995. Acylated cyanidin glycosides in the purple-red flowers of Bletilla striata. *Phytochemistry,* **40**, 1523–1529.

Saito, N., Toki, K., Uesato, K., Shigihara, A., Honda, T., 1994. An acylated cyanidin glycoside from the red-purple flowers of Dendrobium. *Phytochemistry,* **37**, 245–248.

Saito R., Fukuta N., Ohmiya A., Itoh Y., Ozeki Y., Kuchitsu K. & Nakayama M., 2006, Regulation of anthocyanin biosynthesis involved in the formation of marginal picotee petals in Petunia. *Plant Science,* **170**, 828–834.

Scalliet G., Journot N., Jullien F., Baudino S., Magnard J.L., Channeliere S., Vergne P., Dumas C., Bendahmane M., Cock J.M. & Hugueney P., 2002, Biosynthesis of the major scent components 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene by novel rose *O*-methyltransferases. *Elsevier Science B.V.*, **523**, 113-118.

Servettaz, O. Castelli, D, & Longo, C.P. 1975. *Plant Sci.* Lett. 4: 361.

Solecke D., Boudet A., Kacperska A., 1999, Phenylpropanoid and anthocyanin changes in low-temperature treated winter oilseed rape leaves. *Plant physio. Biochem*, **37**, 491-496.

Soontornchainaksaeng P., Chaicharoen S., Sirijuntaru M. & Kruatrachue M., 2001, *In vitro* studies on the effect of light intensity on plant growth of *Phaius tankervilliae* (Banks' ex L' Herit) Bl. And *Vanda coerulea* Giff, *ScienceAsia*, **27**, 233-237.

Stewart, J.P. 1911. Factors Influencing Yield, Color, Size and Growth in Aplles. Proc. Amer. Soc. Hort. Sci. 8:66.

Takeda, K., Harborne, J.B., Self, R., 1986. Identification and distribution of malonated anthocyanins in plants of the Compositae. *Phytochemistry,* **25**, 1337-1342.

Tanaka, Y., Tsuda, S., Kusumi, T., 1998. Metabolic engineering to modify flower color. Plant Cell Physiol. **39**, 1119–1126.

Tan S.C., 1990, *Green Plant Biochemistry*, Percetakan Dewan Bahasa dan Pustaka. Pg 154-156.

Tatsuzawa F., Saito N., Seki H., Yokoi M., Yukawa T., Shinoda K. & Honda T., 2004, Acylated anthocyanins in the flowers of Vanda (Orchidaceae*). Biochemical Systematics and Ecology,* **32**, 651–664.

Tatsuzawa, F., Saito, N., Seki, H., Hara, R., Yokoi, M., Honda, T., 1997. Acylated cyanidin glycosides in the red-purple flowers of Phalaenopsis. *Phytochemistry,* **45**, 173–177.

Tatsuzawa, F., Saito, N., Yokoi, M., Shigihara, A., Honda, T., 1996a. Acylated cyanidin 3,7,30-triglucosides in flowers of Laeliocattleya cv. Mini Purple and its relatives. *Phytochemistry,* **41**, 635–642.

Tatsuzawa, F., Saito, N., Yokoi, M., 1996b. Anthocyanins in the flowers of Cymbidium. Lindleyana, **11**, 214–219.

Tatsuzawa, F., Saito, N., Yokoi, M., Shigihara, A., Honda, T., 1998. Acylated cyanidin glycosides in the orange-red flowers of Sophronitis coccinea. *Phytochemistry* **49**, 869–874.

Taylor K.L., Brackenridge A.E., Vivier M.A & Oberholster A., 2006, Highe-performance liquid chromatography profiling of the major carotenoids in Arabidopsis thaliana leaf tissue. *Journal of Chromatography*, **1121**, 83-91.

Terahara N. Takeda Y., Nesumi A. & Honda T., 2001, Anthocyanins from red flower tea (Benibana-cha), *Camellia sinensis.phytochemistry*, **56**, 359-361.

Thimann, K.V. & Edmoudson, Y.H. 1949. Arch. Biochem. 22:33.

Ulrychova, M. & Sosnova, V. 1970. *Biol Plant* 12: 231.

Wall, M.E. 1939. *Soil Sci.* 47: 142.

Wells R., 2001, Leaf pigment and canopy photosynthetic response to early flower removal in cotton. *Crop Physiology and Metabolism*, **41**, 1522-1529.

William G.H, 1999, *Introduction to Plant Physiology*, Joh Wiley & Sons, Inc., New York.

Willson, M.F., Whelen, C.J., 1990. The evolution of fruit color in fle-shy-fruited plants. Am. Nat. **136**, 790-809.

Winkel_Shirley B., 2001, Update on flavonoid biosynthesis (Flavonoid biosynthesis. A colourful model for genetics, biochemistry, cell biology & biotechnology). *Plant Physiol*, **126**, 485-493.

Wu S., Watanabe N., Mita S., Ueda Y., Masaaki S. & Ebizuka Y., 2003, Two *O*-methyltransferase isolated from flower petals of *Rosa chinensis* var. *Spontanea* involved in scent biosysnthesis. *Journal of Bioscience and Bioengineering*, **96**, 119-128.

Yokoi, M., 1975. Color and pigment distribution in the cultivars of selected ornamental plants, with special reference to their contribution to the ornamental values of plants. Trans. Fac. Hort., Chiba Univ. 14, 20–28.