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Determination of morphological and physiological aspects of the flowers of selected Sri Lankan underutilized blue flower species

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ABSTRACT

Introductions from under-utilized plants would be an innovative approach in breeding for floriculture. This experiment aimed at determining flower morphology of selected underutilized blue flowering plants from Matara District of Sri Lanka and the flower physiology in terms of pigment type and vacuolar pH. Selected blue flower bearing genotypes of *Commelina benghalensis*, *Clitoria ternatea*, an accession from family Boraginaceae (named as genotype "1"), an accession from family Convolvulaceae (named as genotype "2"), *Ipomoea pes-caprae*, and an accession from family Fabaceae named as *"Welmudumahana"* in *Sinhala* language were used. Morphological characters of the flower, flower pigment content and the vacuolar pH were determined. Blue, white and a mixture of blue and white colour in flowers were observed in *Clitoria ternatea* genotypes. A sack like structure was detected from *Commelina benghalensis* as well. The pigment extracts of all blue flowers turned dark blue with 0.1M NaOH indicating that anthocyanin is present in the extract. The highest anthocyanin content of 5.4 and the highest pH of 5.9 were given by *Clitoria ternatea* blue flower with multiple petals. Genotype with a single blue petal gave a pH of 5.8 and OD of 5.08. In *Clitoria ternatea* white colour genotypes the pH was around 4.7 and OD was zero. The genotype with white and blue colour, the pH was around 5 and the OD was 1.2. The anthocyanin content of the each flower was proportionate to the colour intensity of the petals. The vacuolar pH of 5.7 and OD of 3.75 was recorded from *Commelina benghalensis* and the genotype "2". The genotype "1" also had a vacuolar pH of 5.4 and an OD of 3.15.The pH of 5.2 was given by *Ipomoea pes-caprae* and "*Welmudumahana"* which had petals of both blue and purple colours. In the tested genotypes, intensity of blue colour was associated with comparatively high vacuolar pH. Studying the physiology of the flowers of these species with respect to flower colour would be useful in genetic engineering of crops for blue flowers and other importance.

Keywords: Blue flower colour, floriculture, genotypes, Sri Lanka, underutilized flowering plants, vacuolar pH.

INTRODUCTION

Sri Lanka owns rich plant diversity with 7000 indigenous flora, including 3156 flowering plants of which 894 are endemic to Sri Lanka (Dassanayake and Fosberg 1980; Wijesundara et al., 2012)). Around 6,800 species are native to Sri Lanka (Wijesundara *et al.,* 2012). Among them most of the species are underutilized flowering plants. Floriculture in Sri Lanka is limited to anthurium, orchids, Gerbera etc. Therefore, underutilized species should be explored for introductions of potted plants and cut flowers mainly. Major pigment in flowers is anthocyanin. Anthocyanins are divided into cyanidins and their derivatives that produce colours ranging from red to purple (Griesbach, 1996); Pelargonidins and their derivatives that produce colors ranging from pinkish orange to orange (Iwata, *et al.,* 1979); Delphinidins and their derivatives produce colors from blue to deep red (Asen and Siegelman, 1957). Delpinidin is responsible for blue colour through the expression of *F3'5'H* gene in anthocyanin biosynthesis pathway. A number of important ornamental plants, including anthurium, carnations, chrysanthemums, and roses lack blue flower colour genotypes due to absence of *F3'3'H* gene encoding delphinidin. In Chrysanthemum, an induced *F3H* promoter with *F3'5'H* efficiently induced delphinidin production (Noda *et al*., 2013). Accumulation of delpinidin was achieved in white carnations as well, by expressing a *F3'5'H* gene and a petunia *DFR* gene (Tanaka *et al*., 2009; Chandler and Tanaka, 2007; Tanaka *et al*., 2010). The adjusted vacuolar pH was reported to be affecting the flower colour in hydrangea, petunia, morning glory, orchids and roses in addition to pigment content (Asen and Siegelman, 1957., Yoshida *et al*., 2003., Griesbach, 1996). Five native blue flower colour species were selected in this study. *Commelina benghalensis* was detected in both abundant paddy fields and abundant wetlands. *Welmudumahana* was also detected in marginal lands. *Ipomoea pes-caprae* is a cover crop in plantations. *Clitoria ternatea*, Genotype "1" and Genotype "2" are found in home gardens.

Genotypes were identified through taxonomic information. The objective of the study was to determine the morphological and physiological variation of selected genotypes of blue flower species. Our results will be useful as initial work on introduction of underutilized flowering plants and breeding for blue flower colour in Sri Lankan crops.

MATERIALS AND METHODS

Selection of plants: Five blue colour flowering native plants were selected on abundance from Matara District, Southern Province, Sri Lanka in the agro ecological zone of WL2 where annual rainfall and temperature are 1900 mm and 28°C respectively (Department of Agriculture, Sri Lanka, 2016).

Determination of the Taxonomy and Morphology of the flower: Taxonomy was determined at family or species level based on flower morphology. The flowers with special flower modifications were recorded. Flower colour, number of petals, sepals and anthers were recorded. Position of the ovary was determined.

Confirmation of purple pigment as anthocyanin (Uimari and Strommer, 1998): One gram from flower of each genotype was weighed and from that 0.5 g was chopped and extract was taken in distilled water and 0.1M NaOH was added to the other 0.5g of water extract. Finally colors of the each accession were observed to prove the presence of anthocyanin.

The pH of pigment extract in distilled water as assumed to be representing the vacuolar pH : Five grams of petals from each genotype was chopped in 5ml of distilled water. The pH was measured in pigment extract in distilled water.

OD Value and the Anthocyanin content of the genotypes: One gram from each accession was weighed and dipped in 5ml of Glacial Acetic Acid overnight to get the total anthocyanin in to the extract. Then Absorbance (OD value) was measured at a wave length of 525nm using the spectrophotometer (CT-8600 Chromotech, Taiwan) and the pH was measured. As the reference, pure Glacial Acetic Acid where Absorbance was zero was used.

RESULTS AND DISCUSSION

Observation of the Taxonomy and Morphology of the flower

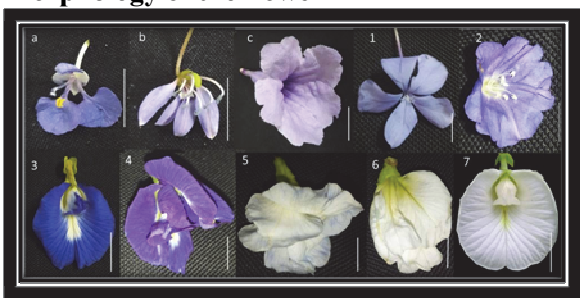

Figure 1. Morphological diversity of selected indigenously grown blue colour flowers in Matara, Sri Lanka. (a) *Commelina benghalensis* (*Diya Meneriya*) from family Commelinaceae, (b) *Welmudumahana* from family Fabaceae (c)*Ipomoea pes-caprae* (*Muhudu Binthambaru*) from family Convolvulaceae, (1) Genotype "1" from family Boraginaceae (2) Genotype "2" from family Convolvulaceae (3), (4), (5), (6) and (7) *Clitoria ternatea (Katarolu)* from family Fabaceae

Ipomoea pes-caprae (*Muhudu Binthambaru*) and Genotype "2" are included in the family Convolvulaceae (Dassanayake, 1980). Flowers of genotype 2 are borne singly or in small clusters. The corolla is round with five petals. Flower has five stamens and length of the filaments variable in

Figure 2. Flowers of *Commelina benghalensis.* (a) Flower, (b) peduncle, (c) the sack at the peduncle base

the same flower. Ovary is superior. Genotype "2" flower characters were mostly similar to the *Evolvulus alsinoides* in tribe Cresseae: *Ipomoea pes-caprae* belongs to tribe Ipomoeeae. This flower has five sepals, five fused petals, five stamens (stamens fused to the petals) and a superior ovary. *Commelina benghalensis is a flower with six* tepals, in two whorls of three each, the outer whorl is green and the inner is usually blue. The flowers have six stamens, in

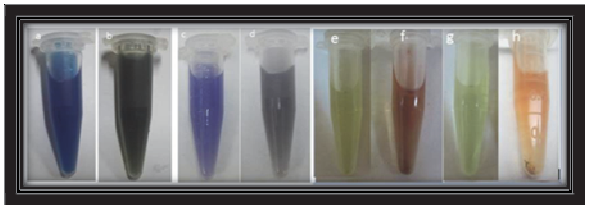

Figure 3. Confirmation of purple pigment as anthocyanin (Uimari and Strommer, 1998). (a)- Pigment extract in water of *Clitoria ternatea,* (b)- Pigment extract of *Clitoria ternatea* in water treated with 0.1M NaOH, (c)- Pigment extract of *Commelina benghalensis*, *Ipomoea pes-caprae* in water, (d)- Pigment extract of *Commelina benghalensis, Ipomoea pes-caprae* in water treated with 0.1M NaOH, e- *Anthurium andraeanum* genotype Red pigment extract in water treated with 0.1M NaOH, f- *Anthurium andraeanum* genotype Green pigment extract in water treated with 0.1M NaOH, g- *Anthurium andraeanum* genotype Red pigment extract in water, h- *Anthurium andraeanum* genotype Green pigment extract in water.

two whorls. Ovary is superior. There is a modified structure at base of peduncle making a sack containing a sap, could be the nectar to facilitate cross pollination although nectar free *Commelina spp* are previously reported (Kaul and Koul, 2012). *Clitoria ternatea* falls in to sub family Faboideae with five sepals, 5 petals and ten stamens where nine of them were fused. Five irregular petals were of descending imbricate aestivation in genotype 3 and 7. One petal was large and covered the other small petals. In the other genotypes, the five petals were almost same in size. "*Welmudumahana*" flowers had five small petals, five free stamens. According to the morphological characters Genotype "1" was identified as a species in family Boraginaceae, where the flowers were similar to the genus *Brunnera* with five sepals, five petals, five stamens and with a superior ovary.

The pigment extracts of all the blue flowers turned dark blue with 0.1M NaOH indicating that anthocyanin is present in the extract according to Uimari and Strommer (1998) (Figure 3). The similar observations were given for *Anthurium andraeanum* genotype "Red" proving the availability of anthocyanin.

Anthocyanin content and Vacuolar pH of genotypes

Table 1. Anthocyanin contents, optical density values and vacuolar pH of the tested flower genotypes

Species	Anthocyanin Content(OD/g)	Vacuolar pH
Ipomoea pes-caprae	3.05	5.2
Genotype "1" in family Boraginaceae	3.15	5.4
Commelina benghalensis	3.75	5.7
Welmudumahana	2.94	5.2
Genotype "2" in family Convolvulaceae	3.78	5.7
Clitoria ternatea genotype " 3"	5.08	5.8
Clitoria ternatea genotype " 4"	5.4	5.9
Clitoria ternatea genotype " 5"	1.2	5.0
Clitoria ternatea genotype "6"	0.0	4.7
Clitoria ternatea genotype "7"	0.0	4.7

The highest anthocyanin content of 5.4 and the highest pH of 5.9 were given by *Clitoria ternatea* blue colour genotype with multiple petals (genotype 4) and the genotype 3 with single blue petal gave a pH of 5.8 and OD of 5.08. In *Clitoria ternatea* white genotypes the pH was around 4.7 and OD was zero and the hybrid genotype with both white and blue colour the pH was around 5 and the OD was 1.2. The anthocyanin content of the each flower was proportionate to the color intensity of the petals. The vacuolar pH of 5.7 and OD of 3.75 was given by *Commelina benghalensis* and the genotype "2". The genotype "1" also had a vacuolar pH of 5.4 and an OD of 3.15. The pH of 5.2 was given by *Ipomoea pes-caprae* and "*Welmudumahana"* wherein petals both blue and purple colours were observed. *Clitoria ternatea, Commelina benghalensis* and genotype "2" should have the delpinidin and other three species with blue and purple colour mix may have both delpinidin and cyanidin. Delpinidin is considered as the anthocyanidin that causes blue colour in flowers. Cyanidin is considered as the anthocyanidin that causes purple colour. It can be suggested that *Clitoria ternatea and Commelina benghalensis* should have higher levels of delpinidin. There are reports on changing flower colour in to blue in a higher vacuolar pH. Although the sepals of hydrangea have only one anthocyanin named delphinidin-3-glucoside, the color displayed varies from red to blue (Asen and Siegelman, 1957) with corresponding changes in vacuolar pH from 3.3 to 4.1 (Yoshida, et al., 2003). The attempts to generate blue roses through the introduction of the flavonoid 3', 5 '-hydroxylase (*F3'5'H*) gene were unsuccessful due to improper pH. Katsumoto, *et al.,* (2007) generated blue roses by placing the *F3'5'H* gene into a genetic background with higher vacuolar pH and high flavonol content. Therefore, the relationship between the blue colour in tested flowers and vacuolar pH can be suggested. Further investigations on physiology of the flowers of these species in terms of flower colour would be useful in genetic engineering of the local flowers for blue colour

REFERENCES

Asen, S. and Siegelman, H.W. 1957. Effect of aluminium on absorption spectra of the anthocyanin and flavonols from sepals of *Hydrangea macrophylla* var. Merveille, *American Society of Horticultural Science.,* **70**:478–481.

Chandler, S. and Tanaka, Y. 2007. CRC critical Reviews of Plant Science., **2**: 19-197.

Dassanayake, M.D. and Fosberg, F.R. 1980. A revised Handbook to the Flora of Ceylon- Complete set. Taylor and Francis US, Department of Agriculture, Sri Lanka, Retrieved on 17th December 2017.

Department of Agriculture, Sri Lanka, Retrieved on 17th December 2016

Griesbach, R.J. 1996. The inheritance of flower color in *Petunia hybrida* Vllm. *Journal of Heredity,* **87**:241–245.

Iwata, R.Y., Tang, C.S. and Kamemoto, H. 1979. Concentration of anthocyanins affecting spathe color in anthuriums. *Journal of American Society of Horticultural Science,* **110**:383–385.

Katsumoto, Y., Fukuchi-Mizutani, M., Kukui, Y., Brugliera, F., Holton, T.A., Karan, M., Nakamura, N., Yonekura-Sakakibara, K., Togami, J., Pigeaire, A., Tao, G.Q., Nehra, N.S., Lu, C.Y., Dyson, B.K., Tsuda, S., Ashikari, T., Kusumi, T., Mason, J.G., and Tanaka, Y. 2007. Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiology*, **48**:1589–1600.

Kaul, V. and Koul, A.K. 2012. Staminal variation and its possible significance in Commelina benghalensis L. and Commelina caroliniana Walter. *Current Science,* **103**:(4):419-425.

Noda, N., Aida, R., Kishimoto, S., Ishiguro, K., Fukuchi-Mizutani, M., Tanaka, Y. and Ohmiya, A. 2013. Genetic engineering of novel bluer-colored chrysanthemums produced by accumulation of delphinidin-based anthocyanins. *Plant and Cell Physiology,* **54:(**10):1684-1695.

Tanaka, Y., Brgliera, F., Kalc, G., Senior, M., Dyson, B., Nakamura, N., Katsumoto, Y. and Chandler. S. 2010. Flower Color Modification by Engineering of the Flavonoid Biosynthetic Pathway: Practical Perspectives. *Bioscience, Biotechnology, and Biochemistry*, **74**(9):170-179.

Tanaka, Y., Brugliera, F. and Chandler, S. 2009. *International Journal of Molecular Sciences,* **10**: 5350-539.

Uimari, A. and Strommer, J. 1998. Anthocyanin regulatory mutations in pea: effects on gene expression and complementation by *R*-like genes of maize. *Molecular Genetics and Genomics,* **257**:198–204.

Wijesundra, D. S. A. 2012. Present Status of Montane Forests in Sri Lanka. *The National Red List 2012 of Sri Lanka: Conservation status of the Fauna and Flora*. BDS Secretariat of the Ministry of Environment and the Department of National Botanic Gardens, Colombo and Peradeniya, Sri Lanka.

Wijesundra, D. S. A., Kathriarachchi, H.S., Ranasinghe, S. W. and Hapuarachchi, G. 2012. Analysis of Seed Plants of Sri Lanka. *The National Red List 2012 of Sri Lanka: Conservation status of the Fauna and Flora*. BDS Secretariat of the Ministry of Environment and the Department of National Botanic Gardens, Colombo and Peradeniya, Sri Lanka.

Yoshida, K., Toyama-Kato, Y., Kameda, K. and Kondo, T. 2003. Sepal color variation of Hydrangea macrophylla and vacuolar pH measured with a proton-selective microelectrode. *Plant Cell Physiology*, **44**:262–268.