



Asian Journal of Plant Sciences

ISSN 1682-3974

science
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A Comparative Study on Selected Antioxidants During Pollination Induced Senescence on *Dendrobium* Sonia and *Dendrobium* Savin White

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Abstract: *Dendrobium* Sonia and *Dendrobium* Savin White are popular *Dendrobium* hybrids in the Orchidaceae Family which are ethylene-sensitive and exhibit earlier senescence due to pollination. Pollinated *Dendrobium* Sonia and *Dendrobium* Savin White showed shorter vase life and initiate earlier perianth senescence that demonstrates rapid sepal and petal wilting and discolouration. Experiments were carried out to observe the changes in antioxidant activities and pigment fading throughout the flower's vase life. As *Dendrobium* Sonia and *Dendrobium* Savin White senesces, there is discolouration, the flower appears dull and weak, which indicates pigment loss and this observation is correlated with the $L^* a^*/b^*$ colour readings carried out using the Chromameter CR-200. The total anthocyanin quantification using the pH-differential method showed that pollinated *Dendrobium* Sonia had lower concentration compared to unpollinated *Dendrobium* Sonia. The total polyphenol assay on pollinated and unpollinated flowers for both hybrids showed lower content in those pollinated compared to those unpollinated. The reducing power assay confirmed that there were antioxidant properties present in both hybrids and the reducing capability increases with the increase of dry weight sample.

Key words: Anthocyanin, folin-ciocalteu, orchidaceae, reducing power, senescence, total phenolic

INTRODUCTION

Orchidaceae is the largest and most diverse of the flowering plant (Angiospermae) family, with over 800 described genera and 25,000 species which occupy wide ranges of ecological habitats and exhibit highly specialized morphological, structural and physiological characteristics (Dressler, 1990). The Orchidaceae Family are considered as the doyens among ornamentals and they are commercially grown globally as cut flowers and potted plants (Martin and Madassery, 2005).

Dendrobium sp. is one of the genera under the Orchidaceae Family and occupies a foremost position in ornamental orchid cut flower industry because of its high number of flowers per inflorescence, a wide range of colour variation and recurrent flowering. *Dendrobium* Sonia and *Dendrobium* Savin White are the most popular *Dendrobium* cut flower hybrid in the market due to its vigour, durability and free flowering characteristics (Fadelah *et al.*, 2001).

Orchid pollinia are known to contain auxin (Stead, 1992) that induces 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity which leads to increased

production of ACC and ethylene (Ketsa *et al.*, 2001). Thus, pollination induced *Dendrobium* sp. from the Orchidaceae family will presumably display senescence earlier as opposed to those which are not pollinated. When ethylene-sensitive flowers like *Dendrobium* sp., are exposed to orchid pollinia which contains auxin (Stead, 1992), which induces 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, leading to increased production of ethylene (Ketsa *et al.*, 2001).

Anthocyanins are one of the major pigments found in *Dendrobium* sp. flowers and are late products of the flavonoid biosynthetic pathway while flavonols like quercetin and kaempferol which are early products, act as co-pigments and form complexes with anthocyanins to enhance and diversify colours in plant tissues (Harborne, 1994).

These flavonoids have low molecular weight organic compounds composed of three-ring structure with various substitutions. The presence of oxy and hydroxyl groups as well as double bonds in specific positions make them strong antioxidants. It is widely believed that antioxidant phytonutrients can inhibit the propagation of free radical reactions that may ultimately lead to the development of

diseases, especially those which are related to aging (Bae and Suh, 2007). Antioxidants are now widely used as ingredients in dietary supplement that are used for health purposes such as preventing cancer and heart diseases (Wang *et al.*, 1999).

Thus, the aim of this study was to investigate the correlation of colour change with pigment loss and to compare total anthocyanin, total polyphenol and reducing power properties in selected antioxidants from *Dendrobium* Sonia and *Dendrobium* Savin White during pollination induced senescence.

MATERIALS AND METHODS

Plant materials: *Dendrobium* Sonia and *Dendrobium* Savin White were purchased from a commercial grower in Old Klang Road, Selangor located about 30 km from the laboratory. Flowers were cut at the pedicels and placed in 10 mL glass vials containing distilled water. Pollination was carried out by using a forcep to remove the anther cap and the pollinia were placed onto the stigma. Flowers were kept at room temperature ($26 \pm 2^\circ\text{C}$) during the period of study.

Perianth colour change: Perianth colour change of flowers were determined by using the Minolta Chroma Meter (CR-200) in $L^* a^* b^*$ co-ordinates in the Munsell Colour System where L^* is Hue; a^* is chroma and b^* is value. Negative a^* values indicate green and higher positive a^* values red colour. Higher positive b^* values indicate a more yellow colour and negative b^* blue colour.

Estimation of total anthocyanin: Total anthocyanin contents of samples were determined using the pH-differential method described by Giusti and Wrolstad (2001).

Preparation of flower extract: Whole flower (50 g) was homogenized and extracted in 100 mL of 70% ethanol for 4 h at room temperature (Lee and Wicker, 1991). The extract was filtered through myra cloth and rinsed with 50 mL of 70% ethanol. Extraction of the residue was repeated using the same conditions. The two filtrates of ethanol were combined and evaporated at 40°C to obtain dry extract. The extracts were placed in 1.5 mL microtubes and stored at -20°C until used.

Determination of total polyphenol standard curve: The standard curve was calibrated using method described by Bae and Suh (2007).

Total polyphenol of flower extract: Total polyphenol content was determined using the Folin-Ciocalteu method

(Bae and Suh, 2007). The absorbance was read at 750 nm and the total polyphenol concentration was calculated from a calibration curve ($r^2 = 0.9875$) using gallic acid as standard.

Reducing power assay: The flower extracts (0.02, 0.04, 0.06 and 0.08 g, respectively) were mixed with 200 μL of phosphate buffer pH 6.5 and 200 μL of potassium ferricyanide (0.01 g mL^{-1}) and incubated at 50°C for 20 min. Two hundred fifty microliter of trichloroacetic acid (0.1 g mL^{-1}) was added to the mixture and centrifuged for 10 min at room temperature (27°C). The resulting supernatant was added into 500 μL of deionized water and 100 μL of ferric chloride (0.001 g mL^{-1}) and incubated at 37°C for 10 min. The absorbance were read at 700 nm. All values shown in the results are average for triplicates carried out.

RESULTS AND DISCUSSION

It was observed that the chromatically determined $L^* a^* b^*$ colour range became negative in *Dendrobium* Sonia and more positive in *Dendrobium* Savin white as the flower senesced. As the perianth discolour, there was a corresponding change in $L^* a^* b^*$ values (Fig. 1, 2). After 11 days, the pollinated *Dendrobium* Sonia flower fully senesced and $L^* a^* b^*$ was measured at a lower negative value of -96.20 as opposed to the unpollinated flower which was measured at -80.48 during full senescence after 24 days. Pollinated *Dendrobium* Savin White flowers fully senesced on day 11 and $L^* a^* b^*$ was measured at a higher negative value of -18.81 as compared to the unpollinated flowers which senesced after day 13 and measured at -27.77.

Total anthocyanin quantification was carried out in both *Dendrobium* hybrids but results were obtained only in *Dendrobium* Sonia. The total anthocyanin on day 0 was measured at $237 \mu\text{g g}^{-1}$ and when the flower senesced, it decreased to $207 \mu\text{g g}^{-1}$ and $218 \mu\text{g g}^{-1}$ for pollinated and unpollinated flowers, respectively (Fig. 3).

The total phenol assay on pollinated *Dendrobium* Sonia showed lower total phenolic compound, $301 \mu\text{g g}^{-1}$, compared to $449 \mu\text{g g}^{-1}$ in unpollinated *Dendrobium* Sonia (Fig. 4). As for *Dendrobium* Savin White extract, results showed that there was $492 \mu\text{g g}^{-1}$ total phenolic compound in its dry weight. Senesced pollinated *Dendrobium* Savin White showed lower total phenolic compound, $244 \mu\text{g g}^{-1}$, compared to $296 \mu\text{g g}^{-1}$ in senesced unpollinated *Dendrobium* Savin White (Fig. 4).

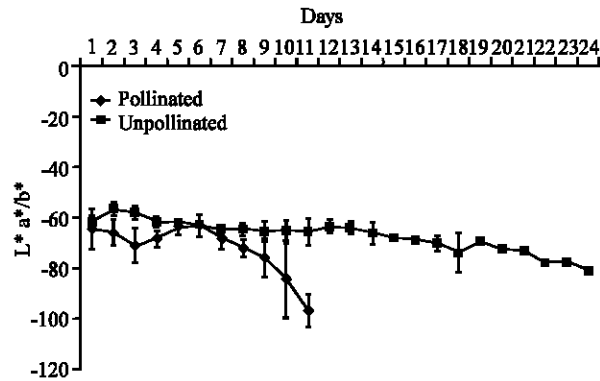


Fig. 1: $L^* a^*/b^*$ Measurements of pollinated and unpollinated *Dendrobium* Sonia during Senescence. Pollinated *Dendrobium* Sonia showed earlier senescence and $L^* a^*/b^*$ measurements showed pollinated flowers exhibit faster and more negative colour change

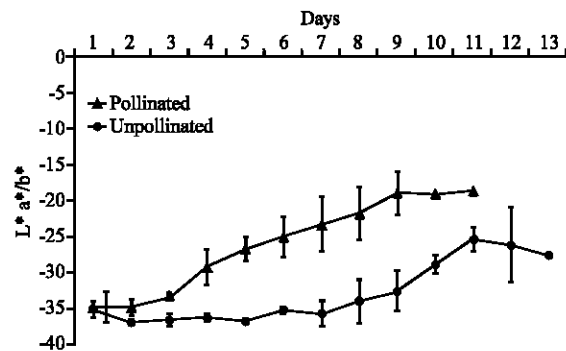


Fig. 2: $L^* a^*/b^*$ Measurements of pollinated and unpollinated *Dendrobium* Savin White during senescence. Pollinated *Dendrobium* Savin White showed earlier senescence and $L^* a^*/b^*$ measurements showed pollinated flowers exhibit faster and more positive colour change

Results showed that the reducing capability of antioxidant in pollinated *Dendrobium* Sonia increases from 0.050 to 0.182 and there was an increase from 0.065 to 0.258 in unpollinated *Dendrobium* Sonia (Fig. 5). Results for pollinated *Dendrobium* Savin White showed that the reducing capability of antioxidant increased from 0.050 to 0.139 while unpollinated *Dendrobium* Savin White showed increment from 0.115 to 0.223 in unpollinated *Dendrobium* Savin White (Fig. 6).

In this study, pollinated flowers of both *Dendrobium* Sonia and *Dendrobium* Savin White showed earlier senescence and signs of wilting including loss of pigments causing the flower to look dull and weak, compared to unpollinated

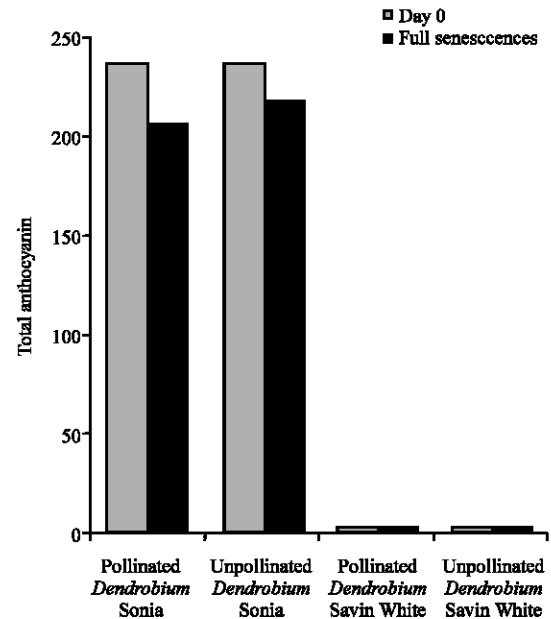


Fig. 3: Total anthocyanin in pollinated and unpollinated *Dendrobium* Sonia and *Dendrobium* Savin White. Results were only obtained from *Dendrobium* Sonia and pollinated *Dendrobium* Sonia showed lower total anthocyanin content when senesced

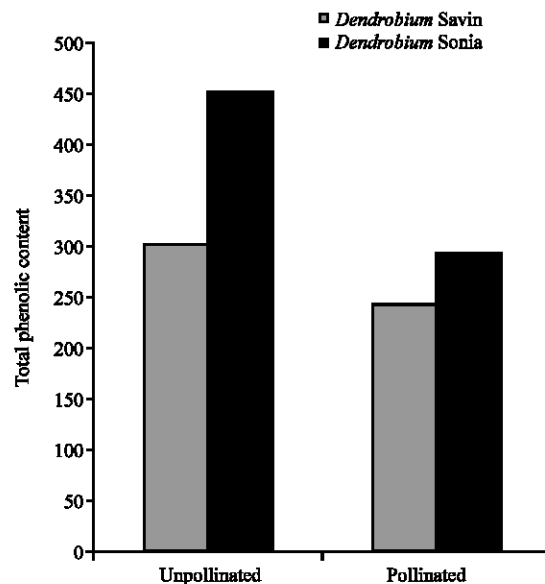


Fig. 4: Total phenolic content of pollinated and unpollinated *Dendrobium* Sonia and *Dendrobium* Savin White. Pollinated and unpollinated *Dendrobium* Sonia showed higher total phenolic content as compared to *Dendrobium* Savin White

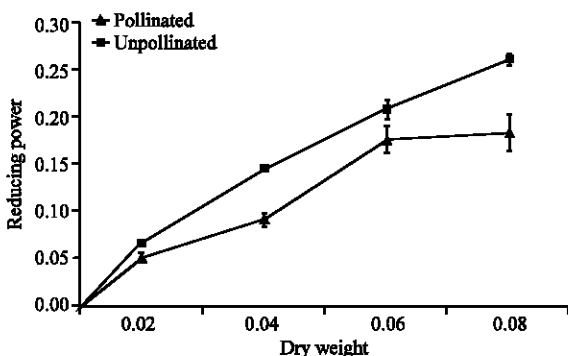


Fig. 5: Comparison of reducing power in pollinated and unpollinated *Dendrobium* Sonia. Reducing capabilities increases with the increase of dry weight sample

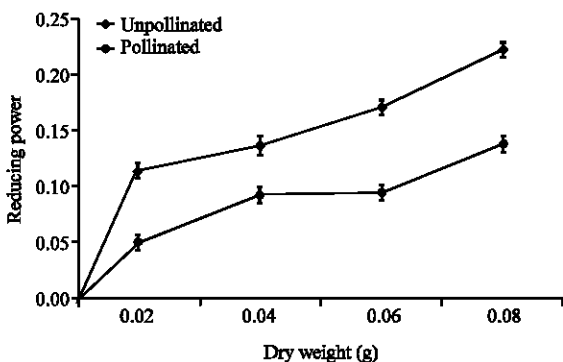


Fig. 6: Comparison of reducing power in pollinated and unpollinated *Dendrobium* Savin White. Reducing capabilities increases with the increase of dry weight sample

flowers which exhibit longer vase life. This corresponds to previous studies (O'Neill and Nadeau, 1997; Doorn, 1997; Chandran *et al.*, 2006) which reported that, pollination of flowers initiates a series of dramatic changes including ethylene production, epinasty, colour fading, abscission, ovary growth and senescence. The senescence phenomena which is hugely characterized by colour fading was studied using visual observation and by objective measurement using the $L^* a^*/b^*$ colour system which showed that as the flowers senesces, there was a change in the colour reading, confirming that there was pigment loss and flowers were discoloured (Fig. 1, 2). It was also noted that at the time of termination, pollinated flowers showed greater colour change (a higher or lower negative reading) compared to the unpollinated flowers, suggesting that there was accelerated physical changes, biochemical changes and deterioration in pollinated flowers.

The total anthocyanin and total polyphenol contents are good indicators of antioxidant capacity and it has been reported a high correlation between antioxidant capacity and total polyphenols (Lako *et al.*, 2007). Flavonols like quercetin and kaempferol which are common pigments in white flowers, have powerful antioxidant capacity and were also selected targets in this study.

Only extracts from *Dendrobium* Sonia gave significant results in the total anthocyanin assay which detects colour for reading and no result were obtained from *Dendrobium* Savin White, probably because quercetin and kaempferol which are white, are the major pigments in *Dendrobium* Savin White (Dubois, 1980). As the flower senesces, the total anthocyanin content decreases (Fig. 3). It was observed that pollinated flowers which were terminated at Day 11 had lower total anthocyanin content compared to unpollinated flowers which were terminated much later at Day 24. This result correlates with the $L^* a^*/b^*$ readings which indicated that pollinated flowers had a more negative value at day of termination and this is supported by Matile (1997), who reported that pollinated senescent petals shows increased leakage of anthocyanins and electrolytes.

There is no method by which total phenolics can be precisely quantitated because of their chemical diversity (Somers and Evans, 1977) and the Folin-Ciocalteu analytical method have been generally preferred when it comes to total polyphenol determination with an arbitrary standard of gallic acid. The total polyphenol content from *Dendrobium* Sonia extract was higher than those in *Dendrobium* Savin White in all both conditions: pollinated and unpollinated extracts (Fig. 4).

Lako *et al.* (2007) reported on total polyphenol contents of a variety of common fruits in human diet, there was $260 \mu\text{g g}^{-1}$ in *Carica papaya* (Papaya), $270 \mu\text{g g}^{-1}$ in *Cocos nucifera* (Coconut), $280 \mu\text{g g}^{-1}$ in *Citrus reticulata* (Orange) and $670 \mu\text{g g}^{-1}$ in cheery. Comparing the result from this study to the findings of Lako *et al.* (2007) the total polyphenol content from *Dendrobium* Sonia and *Dendrobium* Savin White were reasonably significant and could be a source of polyphenol if consumed through daily diet or pharmaceutical products.

The reducing power method established by Bae and Suh (2007) employed ferric chloride (FeCl_3) as an oxidant. The ferrous ion produced from the redox reaction forms a coloured product with trichloroacetic acid. The reducing properties are generally associated with the presence of reductones (Kumaran and Karunakaran, 2007), which exert antioxidant action by breaking the free radical chain reaction by donating a hydrogen atom.

It was observed that both *Dendrobium* Sonia and *Dendrobium* Savin White in pollinated and unpollinated extract showed that the reducing power increases with the increasing amount of dry weight sample. The results for this assay showed that the reducing power extract from *Dendrobium* Sonia had stronger antioxidant activities compared to *Dendrobium* Savin White (Fig. 5, 6). This could be due to the presence of anthocyanins in *Dendrobium* Sonia which is more widely distributed in plants (Gould and Quinn, 1999; Lee and Collins, 2001) compared to flavonols which most of the time are by-products. This can also be correlated back to the total phenolic determination results where there was higher phenolic content in *Dendrobium* Sonia compared to *Dendrobium* Savin White. Nevertheless, the results reflect that both the *Dendrobium* hybrids have reducing capability and the tested extracts are likely to contribute significantly towards having antioxidant effects.

CONCLUSION

This study showed that as *Dendrobium* Sonia and *Dendrobium* Savin White senesces there was visible discolouration, pigment leakage and decreased antioxidant properties. In all the results obtained, extracts of unpollinated *Dendrobium* Sonia had higher total phenolic content, significant total anthocyanin content and higher reducing power capabilities. The better source for higher antioxidant content would be *Dendrobium* Sonia but anthocyanins and flavonols are antioxidants with different reactions and targets owing to different benefits. Although additional data are needed to better understand the antioxidant activities in this two *Dendrobium* hybrids, the results of this study confirms that extract from both flowers represent a significant source of phenolic (especially anthocyanin and flavonol) antioxidants. Further studies on the bioavailability, absorption, metabolism and pharmacokinetics of these antioxidants have to be carried out to ascertain the possible health benefits that can be derived from *Dendrobiums*.

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