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Morphological and biochemical changes in pollinated flowers of different *Aerides* species

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Abstract

Orchid flowers are weird for having prolonged life span; though they may stay fresh in an un-pollinated state for few weeks, they show rapid senescence upon pollination. *A. odoratum* Lour. *A. crispa* Lindl., *A. maculosum* Lindl. *and A. ringens* Lindl. (Fisher.) flowers were stay fresh for 17 days (d), 15d, 13d and 11d respectively. Their pollinated flowers become senescent at 5 days after pollination (DAP), 4d, 12 Hour after pollination, 4DAP and 3d, 12HAP respectively. Pollinated flowers had higher contents of sugars and free amino acids than unpollinated flowers in all species studied. The amount of sugars and activity of enzymes were relatively greater in the pollinated flowers of *A. odoratum* followed by *A. crispa, A. maculosum* and *A. ringens*, reflecting their faster rate of senescence compared to unpollinated flowers. Among floral organs, column and ovaries had relatively lower level of sugars and enzymes compared to lip and perianth at unpollinated stage, although former tissues showed greater contents of simple molecules in the pollinated flowers. It demonstrates signals related to pollination had up-regulated enzymatic activities and mobilization of simple molecules from the lip and perianth to the column and ovary. An increase in anthrocyanin contents and decrease in carotenoid level were found in wilted pollinated flowers which lead to darkening of lips in all species.

Keywords: Post-pollination, *Aerides*, orchid, floral organs, senescence.

INTRODUCTION

Orchids are widely grown as greenhouse or indoor decorative flowering plants [1]. Although orchid flowers vary in shape, colour and size, they share a common configuration of three petals and three sepals [2]. The bottom petal known as labellum (lip) is conspicuously shaped, bigger in size with brighter colour patterns than the top petals. Perianth of orchid flowers is showy and colourful which increases its ornamental value [2]. Orchid flowers can stay fresh for 10 days- 3 months at unpollinated stage [3]. Because of horticultural importance of perianth, a majority of the research is focused on this phase of flower development. *Aerides* genus has white flowers with purple or pink edges. Different *Aerides* species flowers can be discriminated based on size of flowers, brightness and shade of purple/ pink colour.

Pollination regulates developmental responses that contribute to successful sexual reproduction in higher plants. Perianth senescence is the most visible manifestation of pollination- regulated flower development. Pollination also results in wilting and abscission of the sepals and petals which decreases commercial value of flowers [4]. Pollination can induce diverse patterns of pigmentation changes including colour fading, enhanced pigmentation, or intensification of pigmentation in discreet spots. In *Aerides*, very little is known about the response of various floral organs to the pollination at the morphological and biochemical level, and also about how these organs are related to each other in pollinated flowers. The aim of this study is to determine post- pollination morphological and biochemical changes in four *Aerides* species: *A. crispa, A. maculosum, A. ringens,* and *A. odoratum.* This study focuses on the broader context of pollination-regulated consequences of morphological and biochemical changes that collectively lead to the shedding of some floral organs and prepare other organs for fertilization, embryogenesis and fruit development.

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MATERIAL AND METHODS

Orchid species [*Aerides crispa*, *A. maculosum, A. ringens* and *A. odoratum*] were collected from their natural habitats and were maintained in the 'Orchid house', Department of Botany, Bangalore University, Bangalore, India. Their flowers were hand-pollinated at 9:00 a.m. and examined daily twice at an interval of 12 hours for visible morphological changes. Pollinated flowers were harvested just before the shrinkage. Unpollinated flowers were collected after 5 days of their bloom. Both pollinated and unpollinated flowers were dissected in two parts lip- perianth and column- ovary. Morphological changes like pollen germination, change in lip, perianth, column and ovary were observed by naked eyes. [5 - 6]

Determination of weight and relative water content

The fresh weight and dry weight of pollinated and unpollinated flowers were determined with help of electronic balance. Weight of mature fruits (pods) were also measured with the help of electronic

balance [7]. Relative water content was calculated by using the formula: fresh weight - dry weight/ fresh weight X 100.

Alpha amylase enzyme activity

It was determined by modified starch- iodine method described by Fuwa [8]. Flower parts (0.5 gram) were torn into small pieces and homogenized in 5.0 ml of 0.2M phosphate buffer (ph 8). In this reaction mixture 5ml of 1% starch solution of phosphate buffer (ph 7) was added and incubated at 37°C for 30 minutes. Iodine reagent was prepared by mixing 0.5 gm of iodine and 1gm of potassium iodide in 100 ml of water. A 1 ml aliquot of iodine reagent was added to the reaction mixture and optical density was measured using spectrophotometer at wavelength 620. Blank was prepared by using reaction mixture lacking flower extract. Standard graph was prepared with dilutions of starch from 200 µg/ml to 1000 µg/ml. Presence of α-amylase was calculated based on inhibition of α-amylase to react starch with iodine by using following equation:

$$\text{Inhibition of } \alpha\text{-amylase (\%)} = \frac{\text{Abs}_{(sample)} - \text{Abs}_{(control)}}{\text{Abs}_{(sample)}} \times 100$$

Determination of β-Amylase activity

Enzymatic activity of pollinated and unpollinated flowers was assayed as described by Bernfeld [9]. The reaction mixture was prepared containing 1ml of enzyme extract (0.016 M Sodium acetate, pH 4.8) and 1.0ml of freshly prepared starch solution. It was incubated at 25°C for 1 h. The reaction was terminated by adding 1.0 ml DNSA. Afterward, the tubes were kept in boiling water for 10 min, and then cooled at room temperature. The absorbance was recorded at 560 nm. The reference curve was prepared with a standard maltose solution.

Determination of invertase activity

Invertase activity was assayed [10] by extracting flowers in 0.6 ml of 0.2 M acetate buffer (pH 4.8). It was combined with 0.3 ml of 0.4 M sucrose solution and incubated at 30°C for 30 min. After that, 1 ml of DNSA reagent was added to this reaction mixture. The tubes were placed in a boiling-water bath for 10 min, and then cooled to room temperature. Absorbance was recorded at 560 nm, and the reference curve was prepared with a standard glucose solution.

Determination of protease activity

Protease activity was estimated according to the method of Salmia et al. [11] with few modifications. Tissues were homogenized in 5 ml of 0.1 M phosphate buffer (pH 6.0) and spun at low temperature in a high speed centrifuge. The supernatant was used as enzyme extract. A mixture containing 0.5 ml of casein solution (1% in 0.1 M phosphate buffer, pH 6.0) and 0.5 ml of enzyme extract was incubated at 40°C for 1 h before the reaction was terminated by adding 0.1 ml TCA (40%). The mixture was centrifuged and TCA soluble components were retained. Folin-phenol reagent was added to it and absorbance of the reaction mixture was read at 620 nm.

Estimation of Anthocyanins

Pollinated and unpollinated floral organs were homogenized in 5 ml of methanol containing 1 N HCl (9:1 methanol: HCl) and maintained at 4°C for overnight. Particulates were removed by centrifuging the homogenate at 10,000 g for 30 min. Absorbance of the clear supernatant was read at 530 nm and expressed on a per-gram basis [12].

Estimation of Carotenoids

Carotenoids were estimated in pollinated and unpollinated flowers as the method described by Holm [13]. Tissues were homogenized in a clean mortar for 5 min in 5 ml of 80% acetone. The process was repeated twice in 5 ml of 80% acetone. All three extracts were combined and filtered for full recovery of all carotenoids. Optical density was measured using spectrophotometer at wavelength 470, 663 and 646 nm. Concentration of carotenoid was calculated by using Lichtenthaler and welburn equations [14].

Experimental design and statistical analysis

The experiments were set up in completely randomized design. All the experiments were repeated thrice. The data of all the experiments were subjected to one–way analysis of variance (ANOVA) using SPSS (SPSS Inc., IL, USA) [15].

RESULTS AND DISCUSSION

Four *Aerides* species: *A. crispa, A. maculosum, A. ringens* and *A. odoratum* flowers were found different in their life span at both pollinated and un-pollinated conditions. Unpollinated flowers of *A. odoratum* were stay fresh for 17 days (d), followed by *A. crispa, A. maculosum,* and *A. ringens* for 15d, 13d and 11d respectively. Pollinated flowers attained senescence much earlier then unpollinated. *A. odoratum* become senescent at 5 days after pollination (DAP), *A. crispa* at 4 days and 12 hours after pollination (HAP), *A. maculosum* at 4 DAP and *A. ringens* at 3 days and 12 HAP. Pollen germination occurred within 24h for all four *Aerides* species studied. Floral lips and corolla began to fade at 2-3 DAP in *A. ringens*, 3-4 DAP in *A. maculosum* and *A. crispa* and at 4 to 5 DAP in the *A. odoratum*. Wilting began at 1.5 DAP in *A. ringens,* 2 to 3 DAP in *A. maculosum,* 3 DAP in *A. crispa* and at 4 DAP in *A. odoratum* in pollinated flowers. The same starts in unpollinated flowers of *A. crispa, A. maculosum, A. ringens* and *A. odoratum* at 12d, 10d, 9d and 14d respectively (Table. 1). Post pollination changes in column and ovary could be observed within 24 hours, of completion of the process of pollination in *A. maculosum* and *A. crispa*. *A. odoratum* illustrated all these events one day after pollination and *A. ringens* conformed changes at 12-24 hour interval. Similar effect was observed by Chaturvedi and Chaturvedi [6].

Early wilting and darkening of lip and perianth, loss of fresh weight of flowers, with reduced relative water contents were among most easily detectable post pollination incident in all four species flowers. Pollinated ovaries reached to its maximum size at 30-40 DAP. Maximum size and weight of ovary was least in *A. ringens* preceded by *A. maculosum, A. crispa* and *A. odoratum* respectively (Table. 1). One discriminative explanation for this is losses of water in the floral organs which wilt and gains in those which swell is a time dependent process [16, 17].

We studied the relative effectiveness of enzymatic activities and biomolecules in inducing the post-pollination phenomena in four *Aerides* species. Our biochemical assays were found higher enzyme levels in pollinated flowers then unpollinated ones. An increase in the activities of α-amylase (Table 2) and β-amylase (Table 3), two enzymes involved in the hydrolysis of starch, as well as invertase (for sucrose; Table 4) and proteases (proteins; Table 5) in pollinated flowers might be due to up-regulation of pollination-related signals which cause degradation of complex molecules like starch and proteins to simple molecules during senescence. The amount of total sugars, amino acids and the activity of enzymes were relatively greater in the pollinated flowers of *A. odoratum* when compared with other species, reflecting the faster rate of senescence in that species. Sugars, amino acids and enzyme activities were noticed least in unpollinated flowers of *A. ringens* leaded by unpollinated *A. maculosum, A. crispum* and *A. odoratum*. The rise in sugar concentrations of pollinated flowers may serve as a critical source of energy during programmed cell death [18]. Ovaries and column had relatively lower levels of sugars and enzymes compared with lip and perianth in unpollinated stage, although former tissues showed greater contents of simple molecules in the pollinated flowers. It suggest that, upon pollination a signal may emanate from the pollen itself or be induced by metabolic changes which demonstrate mobilization of free molecules from the lip and perianth to the column and ovary. Early senescence of lip and perianth of pollinated flowers may be linked to sugar starvation because the exogenous application of those molecules seems to prolong the floral life span [19]. Similar studies conducted on other orchid species, shows an increase in sugar level in the gynostemia and perianth segments of *Cattleya* sp. flowers but not of *Cymbidium* [17]. These metabolic alterations appear to be part of a general floral response during the organized degenerative process of senescence, involving several hydrolytic reactions that break down more complex molecules [19].

Table.1 Comparison of post pollination morphological changes in floral organs

Event	*A. crispa*	*A. maculosum*	*A. ringens*	*A. odoratum*
Fresh weight of unpol	240 mg	210 mg	180 mg	350 mg
Fresh weight of pol	180 mg	165 mg	120 mg	300 mg
Dry weight of unpol	60 mg	50 mg	20 mg	70 mg
Dry weight of pol	50 mg	45 mg	20 mg	55 mg
Relative water content in unpol flower	75%	76%	89%	84%
Relative water content in pol flower	72%	73%	83%	82%
Senescence in unpol	15 days	13 days	11 days	17 days
Senescence in pol	4d and 12HAP	4 DAP	3d and 12HAP	5 DAP
Change in lip	1 DAP	Within 24 h after pol.	12- 24 h after pol.	1 DAP
Initiation of increase in column size	Within 24 h after pol.	Within 24 h after pol.	12- 24 h after pol.	1 DAP
Initiation of increase in diameter of ovary	1 DAP	Within 24 h after pol.	12- 24 h after pol.	1 DAP
Pollen germination	Within 24 h after pol.	Within 24 h after pol.	Within 24 h after pol.	Within 24 h after pol.
Maximum diameter of ovary	4 cm	3.5 cm	2 cm	4.5cm
Maximum length of ovary	2.5 cm	2.2 cm	2 cm	3 cm
Weight of mature fruit	175 mg	120 mg	55 mg	210 mg
Perianth wilting of unpol. (just before shrinkage)	12 days	10- 11 days	9 days	14 days
Perianth wilting of pol. (just before shrinkage)	3-4 DAP	3 DAP	Within 3 DAP	4 DAP

Note: Pol: Pollinated flowers; Unpol: unpollinated flowers; HAP: hours after pollination; DAP: Days after pollination

Table.2: α-amylase activity (μg starch hydrolyzed $0.5g^{-1}$ fw) in floral organs of four different Aerides species

Species	Pol Column, Ovary	Pol Lip, Perianth	Unpol Column, Ovary	Unpol Lip, Perianth
A. crispa	450±9.8	220±6.81	140±16.6	200±17.5
A. maculosum	480±15.6	280±11.1	170±13.5	220±15.1
A. ringens	340±10.0	250±7.9	380±7.7	200±5.8
A. odoratum	750±18.2	400±12.7	300±18.8	380±11.6

All the data is mean± SD (p value< 0.05)

Table.3: β-amylase activity (μg reducing sugar formed $0.5g^{-1}$ fw) in floral organs of four different Aerides species

Species	Pol Column, Ovary	Pol Lip, Perianth	Unpol Column, Ovary	Unpol Lip, Perianth
A. crispa	550±11.1	390±12.2	190±12.9	345±10.9
A. maculosum	340±17.5	280±9.1	221±4.9	250±11.5
A. ringens	280±7.22	210±9.1	196±6.1	220±8.2
A. odoratum	650±11.9	400±12.4	296±7.8	380±5.7

All the data is mean± SD (p value< 0.05)

Table.4: Invertase activity (μg reducing sugar formed $0.5g^{-1}$ fw) in floral organs of four different Aerides species

Species	Pol Column, Ovary	Pol Lip, Perianth	Unpol Column, Ovary	Unpol Lip, Perianth
A. crispa	720±15.5	680±8.8	250±12.5	370±11.1
A. maculosum	500±18.5	430±7.0	200±17.3	300±11.6
A. ringens	450±19.9	380±11.6	170±16.7	250±12.3
A. odoratum	730±5.9	680±11.1	350±13.6	420±17.9

All the data is mean± SD (p value< 0.05)

Table.5: Amounts of total amino acids (µg 0.5 g−1 fw) in floral organs of four different Aerides species

Species	Pol Column, Ovary	Pol Lip, Perianth	Unpol Column, Ovary	Unpol Lip, Perianth
A. crispa	80±7.8	48±4.8	35±11.1	50±4.6
A. maculosum	65±6.7	35±4.8	27±6.7	30±7.5
A. ringens	45±5.9	30±7.8	20±11.0	21±5.9
A. odoratum	84±9.0	52±9.8	37±11.4	48±7.4

All the data is mean± SD (p value< 0.05)

Table.6: Anthocyanin contents (ΔOD wt/g fw) in floral organs of four different Aerides species

Species	Pol Column, Ovary	Pol Lip, Perianth	Unpol Column, Ovary	Unpol Lip, Perianth
A. crispa	1.37±0.3	1.06±0.06	0.73±0.6	0.98±0.09
A. maculosum	1.17±0.07	0.706±0.04	0.63±0.11	0.88±0.08
A. ringens	0.490±0.08	0.46±0.006	0.29±0.008	0.39±0.006
A. odoratum	0.425±0.1	0.292±0.09	0.81±0.04	0.253±0.012

All the data is mean± SD (p value< 0.05)

Table.7: Amounts of Carotenoid (ΔODwt/0.5g fw) in floral organs of four different Aerides species

Species	Pol Column, Ovary	Pol Lip, Perianth	Unpol Column, Ovary	Unpol Lip, Perianth
A. crispa	0.35±0.03	0.31± 0.02	0.42±0.07	0.55±0.04
A. maculosum	0.39±0.06	0.35±0.08	0.49±0.01	0.590±007
A. ringens	0.60±0.06	0.52±0.09	1.00±0.08	1.20±0.008
A. odoratum	0.21±0.5	0.14±0.09	0.65±0.006	0.85±0.009

All the data is mean± SD (p value< 0.05)

The first detectable symptom, darkening of the lips, was a result of elevated anthocyanin contents and reduced carotenoid contents in pollinated flowers (Table 6, 7). This change in colour continued until the perianth wilted and shrank to its minimum size. These observations agree with Arditti *et al*. [20], and Woltering [21, 22], who reported altered pigmentation, especially enhanced anthocyanin levels, in the perianth and lip of pollinated *Cymbidium* species. Pigmentation changes in *Vanda* orchid flowers are also associated with pollination and ethylene production but, in contrast with *Cymbidium, Vanda* flowers undergo rapid colour fading [23]. The change in pigments presumably serves as a signal to pollinators that flower has been visited or pollinated [24]. Production of anthocyanins and other pigments by columns and lips [25], as well as loss of turgidity and colour changes of perianth were found in many orchid species [26, 27]. Relatively little is known about the specific biochemical events that underlie the process of floral pigmentation changes, although certain floral organ pigmentation changes have been associated with carotenoid or anthocyanin biosynthesis [26], anthocyanin degradation [28], and/ or changes in tissue pH [29].

CONCLUSION

Aerides flowers are valuable because of its beautiful long lasting flowers. This study provides details of pollination-related morphological and biochemical changes which causes flower senescence in orchids. It provides a basic understanding about some of the finer regulatory mechanisms that underlie pollination-related flower senescence in orchids.

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REFERENCES

[1] Griesbach R.J. 2003. Orchids emerge as major world floral crops. *Chronica Horticuture*, 43: 6-9.

[2] Rao T.A., & Sridhar S. 2007. Wild orchids in Karnataka- A pictorial Compendium, first edition. Institute of Natural Resources conservation Education, Research and Training, Bangalore.

[3] O'Neill S.D. 1997. Pollination regulation of flower development. Annual Review, *Plant Physiology and Molecular Biology*, 48: 547-574.

[4] O'Neill S.D. & Nadeau J.A. 1997. Post pollination flower development. Horticulture. Review 19: 1-58.

[5] Attri L.K., Nayyar H., Bhanwra R.K. & Pehwal A. 2008. Post-pollination changes in the floral organs of two Cymbidium species. *Biologia Plantarum.* 52 (4): 787-791

[6] Chaturvedi S.K. & Chaturvedi S. 2010. Biotic Pollination in *Aerides odorata* Lour. (Orchidaceae). *The International Journal of Plant Reproductive Biology*. 2(1): 45-49.

[7] Khondker M., Hassan A., Md. Alfasane A., & Shhjadee F.U. 2010. Flowering and fruiting characteristics and biochemical composition of an endangered palm species (*Corypha taliera* Roxb.). *Bangaladesh J. of Taxonomy,* 17(1): 79-86.

[8] Fuwa H. 1954. A new method of microdetermination of amylase activity by the use of amylase as the substrate. *J. Biochemistry*, 41: 583–603.

[9] Bernfeld P. 1955. Amylases: α and β. In: Methods In: Enzymology, eds, Colowick, S.P. and Kaplan, N.O., Academic Press, New York, p 149-158.

[10] Nygaard P. 1977. Utilization of exogenous carbohydrates for the growth and starch synthesis in pine pollen suspension cultures. *Physiolgia Plantarum*, 39: 206-210.

[11] Salmia M.A., Nyman S.A., & Mikola J.J. 1978. Characterization of proteinases present in germinating seeds of scots pine, *Pinus sylvestris*. *Physiolgia Plantarum*, 42: 252-256.

[12] Sims D.A., & Gamon J.A. 2002. Relationship between pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment*, 81: 337-354.

[13] Holm G. 1954. Chlorophyll mutation in barley. *Acta Agriculturae Scandinavica*, 4: 457-471

[14] Lichtenthaler H.K. & Wellburn A.R. 1983. Determinations of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. *Biochemical Society Transactions*, 11: 591 - 592.

[15] Duncan D.B. 1955. Multiple range and multiple f- tests. *Biometrics*, 11: 1-42.

[16] Hubert B., & Maton J. 1939. The influence of synthetic growth-controlling substances and other chemicals on post-floral phenomena in tropical orchids. *Biologisch Jaarboek*, 6: 244.

[17] Hsiang T.T.H. 1951. Physiological and biochemical changes accompanying pollination in orchid flowers. I. General observations and water relations. *Plant Physiology*, 26: 441-455.

[18] Van Doorn W.G. 2004. Is petal senescence due to sugar starvation? *Plant Physiology*, 134: 35-42.

[19] Van Doorn W.G. & Woltering E.J. 2004. Senescence and programmed cell death: Substance or semantics? *J. Expeimental Botany*, 500: 2147-2153.

[20] Arditti J., Hogan. N.M., & Chadwick A.V. 1973. Post-pollination phenomena in orchid flowers, IV: Effects of ethylene. *American J. Botany*, 60: 883-888.

[21] Woltering E.J. 1990. Inter-organ translocation of 1-aminocyclopropane-1-carboxylic acid and ethylene coordinates senescence in emasculated *Cymbidium* flowers. *Plant Physiology*, 92: 837-845.

[22] Woltering E.J. 1990. Inter-relationship between the different flower parts during emasculation-induced senescence in *Cymbidium* flowers. *J. Experimental Botany*, 41: 1021-1029.

[23] Akamine E.K. 1963. Ethylene production in fading *Vanda* orchid blossoms. *Science*, 140: 1217–1218.

[24] Seshagiriah K. N. 1941. Physiology of pollination in Orchidaceae. *Curv. Science*. 10: 30.

[25] Arditti J., Flick B., & Jefferey, D.C. 1971. Post-pollination phenomena in orchid flowers. 11: Effects of abscisic acid and its interactions with auxin, gibberellic acid and kinetin. *New phytologist*, 70: 333.

[26] Arditti J., & Knauft R. 1969. The effects of auxin, actinomycin D, ethionine, and puromycin on postpollination behaviour by *Cymbidium* (Orchidaceae) flowers. *American J. Botany*, 56: 620.

[27] Mohan Ram H.Y. & Mathur G. 1984. Flower colour changes in *Lantana camara. J. Experimental Botany*, 35: 1656–62.

[28] Proctor J.T.A. & Creasy L.L. 1969. An anthocyanin- decolorizing system in florets of *Cichorium intybus. Phytochemistry,* 8: 1401–3.

[29] Asen S., Stewart R.N., & Norris K.H. 1977. Anthocyanin and pH involved in the colour of 'Heavenly Blue' morning glory. *Phytochemistry*, 16: 1118–19.