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Probing visual and physiological alterations following pollination and enhancing shelf life in some ornamental orchids

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Abstract
The orchids have contributed immensely to the international trade in floriculture due to their beautiful lustrous foliage and long lasting flowers of myriads shapes, sizes and colors. Though, the orchids represent one of the largest families of the flowering plants as state above, but orchid based floriculture has revolved around not more than 100 species in 10-12 genera. Presently, morphological and physiological changed have been documented in pollinated and un-pollinated flowers. The potential inhibitors namely TIBA (inhibitor of Auxins) and silver nitrate (Ethylene inhibitor) have also been used to enhance shelf life by delaying senescence related events. First visible change after pollination was observed in lip colour followed by wilting and swelling of ovary; start from 24 hrs following pollination. Shelf life of the flowers have been enhanced by 10-12 days using inhibitors of auxin and ethylene and ethylene found to be more pronounced effect in delaying senescence to enhance shelf life in taxa under investigations.

Keywords: orchids, flowers, shelf life, inhibitors, pollination

Introduction

Orchids, known for their myriad of shapes, colors and size embody an order of aristocracy among the flowering plants. These extraordinary plants with a spectrum of floral characteristics and intricate pollination mechanisms represent a fairly young (geologically), highly diverse and successful family, Orchidaceae, which is perhaps the largest family of angiosperms with an estimated 28,000 species [1, 2, 3]. The orchids have out-maneuvered their counterparts by evolving ingenuity and higher levels of specialization in both the vegetative and reproductive traits. Orchids still are continue to be in an evolutionary flux due to (i) poorly developed barriers of reproductive isolation, which promote free gene flow across the taxonomic limits and (ii) high survival frequency of neotypes. Nearly 1, 00, 000 natural and manmade hybrids, at levels from interspecific to pleurigeneric have so far been listed [4] and many more are in the offing.

The orchids have contributed immensely to the international trade in floriculture due to their beautiful lustrous foliage and long lasting flowers of myriads shapes, sizes and colors. Though, the orchids represent one of the largest families of the flowering plants as state above, but orchid based floriculture has revolved around not more than 100 species in 10-12 genera. It has been observed that the un-pollinated flowers remain fresh for longer time but senesced immediate after pollination, thus decreasing commercial value. Moreover, the changed following pollination are not been studies in details previously. The present study was aimed at to probing visual and physiological changes following pollinated and un-pollinated flowers of four orchid species varying in life span (Figure 1A-D, Table 1). The potential inhibitors of Auxins and Ethylene have also been used so as to enhance shelf life by delaying senescence related events. To identify some common markers of floral senescence, if any, amongst different orchid species.

2. Material and Methods

The orchids under investigation i.e. *Cymbidium pendulum* and *C. aloifolium* from North Eastern areas (mainly Sikkim) whereas *Rhynchostylis retusa* and *Aerides multiflorum* from Kangra and Palampur (H.P.) were brought from their natural habitat (Figure 1A-D) and maintained in the Orchid House, Department of Botany, Panjab University, Chandigarh. The orchid classification as of 1993 [5] has been followed. The flowers were hand pollinated at anthesis. The treatment of TIBA and silver nitrate have also been spray after pollination. The visual changes in the flowers were noted down after periodic interval of time. The flowers were harvested at two stages of pollination for physiological studies and processed as below:

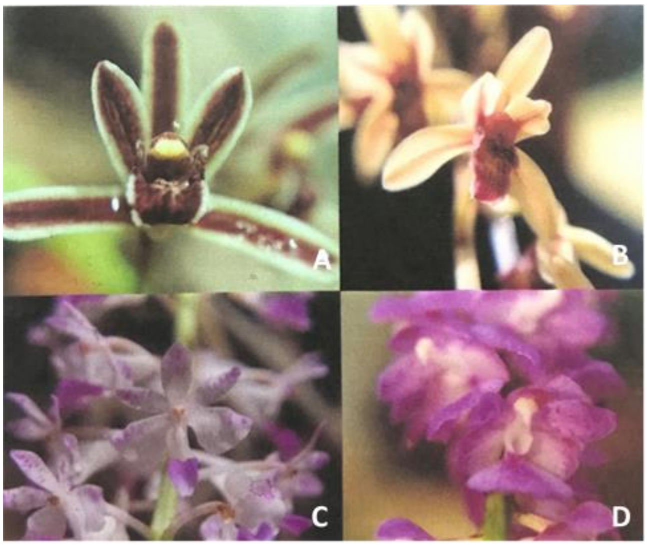

Fig 1: Flowers of taxa under study; (A) *Cymbidium pendulum* (Roxb.) Sw; (B) *Cymbidium aloifolium* (L.) Sw.; (C) *Rhynchostylus retusa* (L.) Bl; (D) *Aerides multiflora* Roxb.

3. Results and Discussion

The various morphological, physiological events occurring in flowers following pollination in all the chosen orchid species having contrasting floral longevity have been observed and were compared with flowers experiencing natural senescence without pollination. Additionally, the pollinated-flowers treated with inhibitors of auxin [2,3,5-triiodobenzoic acid (TIBA), an auxin polar transport inhibitor] and ethylene (silver nitrate), two key molecules universally implicated in regulation of senescence were also assessed for their senescence related changes to examine the involvement of these hormones in regulating the process. We investigated 4 orchid species for this purpose, which differed in their floral longevity as shown (Table 1).

Table 1: Details of floral senescence in pollinated and un-pollinated flowers

Species	Floral Senescence	
	Un-pollinated	**Pollinated**
Aerides multiflorum	17 days	7 days
Cymbidium aloifolium	18	7 days
Cymbidium pendulum	20 days	8 days
Rhynchostylis retusa	24 days	5 days

The findings, in general, revealed that un-pollinated flowers of *Cymbidium pendulum, C. aloifolium* and *Aerides multiflorum* took about 20, 18 and 17 days to show senescence while *Rhynchostylis retusa* showed senescence in 24 days. In contrast, the pollinated flowers attained senescence in 8 days after pollination (DAP) in C. *pendulum*, 7 DAP in C. *aloifolium* and *A. multiflorum* and 5 DAP in *R. retusa* suggesting a rapid progression of senescence related events triggered by pollination (Table 1). These observations are in agreement with some of the earlier ones observed in other orchid species e.g. flowers of *Phalaenopsis* and *Cymbidium* species were found to live up to 8 weeks, if un-pollinated but died within 7 days after pollination. Likewise, *Paphiopedilum* blossoms lasted for 3 months in unpollinated state buts senesced within 3 weeks after pollination [6-11].

The first detectable symptom after pollination was alteration in colour of the lip that became darker in all the species, which occurred due to elevation of anthocyanins that continue to increase till the perianth wilted and shrunk to its minimum size (Figure 2A-I). The change in lip colour happened almost at the same time (i.e. 1 DAP) in all the four taxa. These observations are in line with some previous ones [12-17], which reported pigmentation changes, especially increased anthocyanin levels in the perianth of *Cymbidium* species. In some cases, anthocyanin production and disappearance have been reported [16] while in some others disappearance may follow production [14].

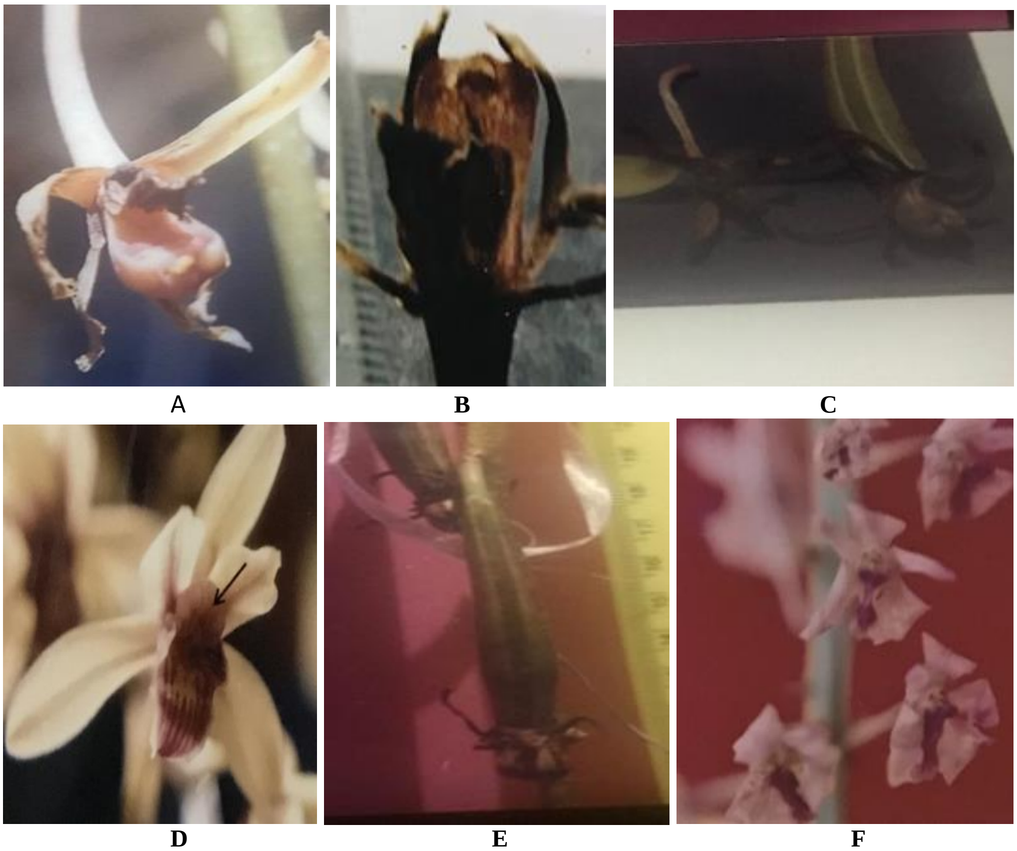

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Fig 2: Morphological changes following pollination (A) Lip color Change and perianth wilting (stage-1) in *C. Pendulum*; (B) Perianth crumpled (stage-II) in *C. Pendulum*; (C) ovary size increase in *C. Pendulum;* (D) Lip colour change and perianth wilting in *C. aloifolium*; (E) Increase of Ovary size in *C. aloifolium*; (F) Peraith wilting in *R. retusa*; (F) Crumpled Perianth and ovary increase in *R. retusa*; (H) Perianth wilting in *A. multiflora*; (I) Crumpled perianth and increases Ovary diameter in *A. multiflora.*

These changes in pigments are suggested to act presumably as a signal to the pollinators indicating that the flower has been visited or pollinated. The underlying specific events affecting floral pigmentation changes are less understood although change in colour has been associated with carotenoid or anthocyanin biosynthesis [18], anthocyanin degradation [19]. Since, we observed distinctive variation in amount of anthocyanins and carotenoids levels at different stages after pollination, diverse biochemical mechanisms appear to be operative in different flowers that require to be explored in detail. It has been suggested that pollination-regulated colour changes evolved independently in angiosperms many different times [20], suggesting that the underlying biochemical mechanisms are likely to differ in taxa. The perianth of flowers in all the species showed wilting following pollination that was one of the quickest symptom observed in pollinated flowers (Figure 2A-I. In *Phalaenopsis* cv. 'Herbet Hager' flowers having longevity of 2-3 weeks showed rapid acceleration of the wilting process, beginning after only 24 hours of pollination [21]. Wilting of the flowers was accompanied by a loss of water from cells of the upper layer of the petals, leading to their upward folding. The findings are in contrast to earlier author [22], who found that pollination did not alter petal and sepal anthocyanin content and fresh as well as dry weight of petals and sepals. But, the fresh and dry weight of stigmas (columns) together with pedicels increased significantly after pollination. Ovary growth of pollinated orchid flowers with petals and sepals intact was greater than that of pollinated orchid flowers without petals and sepals.

3.1. Inter-Organ Relationship

Earlier reports on pollination-induced flower senescence indicate that landing of compatible pollen on the stigma surface triggers the cascade of events leading to senescence [21]. The process is accelerated tremendously with pollination suggesting the involvement of some pollen-released or triggered signal(s) that traverses across the stigma-style, ovary, lip and petals inducing several alterations including senescence. In the present study, we examined the effects of pollination on some visual observations. A change in colour of the lip was the first sign of pollination that occurred in 24 hours in all the species (Table 1). Simultaneously, the column size began to increase within 24 hours accompanied by increase in diameter of the ovary.

These events occurred even before the onset of pollen germination suggesting some signal was released from the pollen. It has been reported earlier that the initial perception of the pollination event is accompanied by as rapid increase in ethylene production by the stigma and style within hours after pollination [23, 24]. In each case, the production of pollen-associated ethylene precedes germination of the pollen tube, suggesting that physical growth of the pollen tube cannot be the primary signal responsible for induction of ethylene biosynthesis [25]. Mock-pollination of orchid flowers with latex beads to the stigma and transmitting tract failed to trigger ethylene production or any other pollination responses, which indicates that physical contact is not a sufficient signal to induce any component of the post pollination syndrome [26]. Considerable attention has been paid to the identification of pollen-borne chemical molecule(s) that are released immediately after pollination. Thus, the potential primary pollen signals include 1-amiocyclopropane carboxylic acid (ACC), auxin, proteins, lipids, pectic oligosaccharides, flavonoids, and methyl jasmonate, all of which have been detected in pollen and some of which are known inducers of ethylene biosynthesis, making them attractive candidates for the primary pollen signal molecule[27, 28]. Several studies indicate that auxins initiate the post pollination developmental events in orchids [12, 15, 29]. The change in column and ovary size observed in our studies has also been associated with pollination-stimulated auxin release. The role of pollination as a primary event in ovary and fruit development has been established in orchids [26]. Previous studies on post pollination development in orchids identified changes in curvature of the ovary to be one of the earliest post pollination events [30]. This change in curvature was more recently shown to be accompanied by the formation of hair cells and cell divisions in the placental ridge that eventually participate in expanding of the placental cavity [26]. It was revealed that pollen germination commenced within 24-48 hours after pollination that coincided with change in lip colour that eventually showed fading [7-11]. Presently, Lip colour started fading in 2 days in *Cymbidium* sp. and 4-5 days in *Rhynchostylis* sp. We examined the pollination effects on floral senescence at two stages-first stage corresponds with initiation of perianth wilting, which occurred at 4-5 days after pollination (DAP) in *Cymbidium pendulum,* after 4-5 DAP in *Cymbidium aloifolium,* 3-4 DAP in *Aerides multiflorum* and 2-3 DAP in case of *Rhynchostylis retusa* while second stage corresponds with complete wilting of perianth, which occurred at 6-7 DAP in *Cymbidium pendulum,* after 5-6 DAP in *Cymbidium aloifolium,* 5-6 DAP in *Aerides multiflorum* and 3-4 DAP in case of *Rhynchostylis retusa.* At second stage, the perianth had begun to show signs of shrinking. Based upon the parameters examined in various organs viz. column, ovary, lip and perianth in all the species, progressive increase in damage to the floral organs was apparent but varied among different organs.

Additionally, application of these inhibitors to pollinated flowers partially suppressed the pollination-induced changes. Silver nitrate had more pronounced effect than TIBA indicating greater involvement of ethylene in mediating pollination caused effects as compared to auxins. Ethylene has been strongly implicated as a potential transmissible secondary signal to other floral parts distal from the stigma that could act to promote a number of post pollination events including perianth senescence. It could be because of larger involvement of ethylene in governing the flower senescence that its inhibitor in our studies proved to be more effectual than auxins inhibitor.

Among the species, *Cymbidium aloifolium* increased its floral life to about 11 days in silver nitrate treated flowers from about 7 days in case of pollinated but untreated flowers. *Cymbidium pendulum* showed increase up to 10 days while *Aerides multiflorum* and *Rhynchostylis retusa* increased the floral, life to 8 and 9 days, respectively with silver nitrate application compared to 7 and 5 days in untreated pollinated flowers. These variations also reflect differential metabolism associated with senescence in these plant species. Sensitivity to ethylene or any other molecule inducing senescence might be one of the reasons associated with variation in senescence time [30, 31].

4. Conclusion

Based upon the parameters examined in various organs viz. column, ovary, lip and perianth in all the species, progressive increase in damage to the floral organs was apparent but varied among different organs. These changes in pigments are suggested to act presumably as a signal to the pollinators indicating that the flower has been visited or pollinated

Ethylene has been strongly implicated as a potential transmissible secondary signal to other floral parts distal from the stigma that could act to promote a number of post pollination events including perianth senescence. It could be because of larger involvement of ethylene in governing the flower senescence that its inhibitor in our studies proved to be more effectual than auxins inhibitor. Conclusively, Silver nitrate application was more effective than TIBA suggesting greater participation of ethylene. These variations also reflect differential metabolism associated with senescence in these plant species.

5. Acknowledgement

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6. References

1. Christenhusz MJM, Byng JW. The number of known plants species in the world and its annual increase. Phytotaxa. 2016; 261(3):201-217.
2. Wida Utami ES, Hariyanto S. Organic Compounds: Contents and Their Role in Improving Seed Germination and Protocorm Development in Orchids. International Journal of Agronomy, 2020, 1-12.
3. Attri LK, Nayyar H, Bhanwra RK. Pollination induced embryological studies in *Aerides multiflora* (Roxb.). International Journal of Botany Studies. 2020; 5(4):211-215.
4. Chadha KL. Orchid floriculture in India. The Journal Orchid Society of India. 1994; 8:1-4.
5. Dressier RL. Phylogeny and classification of the Orchid family. Cambridge Univ. Press, Cambridge, 1993.
6. Arditti J. Fundamentals of Orchid Biology. John Wiley and Sons, New York, 1992.
7. Attri LK, Nayyar H, Bhanwra RK, Vij SP. Post pollination related biochemical changes in floral organs of Rhynchostylis retusa (L.) Bl. and Aerides multiflora Roxb. (Orchidaceae). Journal of Plant Biology. 2007; 50(5):548-556.
8. Attri LK, Nayyar H, Bhanwra RK. Post-pollination changes in the floral organs of two Cymbidium species. Biologia Plantarum. 2008; 52(4):787-791.
9. Attri LK, Nayyar H, Bhanwra RK. Floral Senescence in Two Orchid Taxa: A Post Pollination Biochemical Analysis. Russian Journal Plant Physiology. 2008; 55(6):821-828.
10. Attri LK, Nayyar H, Bhanwra RK, Anju. Pollination induced oxidative stress in floral organs of Cymbidium pendulum (Roxb.) Sw. and Cymbidium aloifolium (L.) Sw. (Orchidaceae): A biochemical investigation. Scientia Hoticulturae./ 2008; 116:311-317.
11. Attri LK, Nayyar H, Bhanwra RK. Pollination induced oxidative stress in the floral organs of Coelogyne cristata Lindl. (orchidaceae): biochemical analysis. Indian Journal of Fundamental and Applied Life Science. 2011; 1(2):68-74
12. Arditti J. Orchids and the discovery of auxin. American Orchid Society Bulletin. 1971; 40:211-214
13. Arditti J. Aspects of the physiology of orchids. Advances in Botanical Research. 1979; 7:421-465.
14. Arditti J, Knauft RL. The effects of auxin, actinomycin D, ethionine and puromycin on post pollination behavior in *Cymbidium* (Orchidaceae) flowers. American Journal of Botany. 1969; 56:620-628
15. Arditti J, Hogan NM, Chadwick AV. Post-pollination phenomena in orchid flowers, IV: effects of ethylene. American Journal of Botany. 1973; 60:883-888
16. Arditti J, Flick BH. Post pollination phenomena in orchid flowers. V. Participation by the rostellum and gynostemium tip. American Journal of Botany. 1974; 61:643-665.
17. Woltering EJ. Initial events and inter-organ relation during senescence of orchid (Cymbidium) flowers. Dissertation, Landbouwuniversiteit, Wageningen, Netherlands, 1990.
18. Mohan Ram HY, Mathur G. Flower colour changes in Lantana camara. Journal of Experimental Botany. 1984; 35:1656-62.)

19. Procter JTA, Creasy LL. An anthocyanin-decolourizing system in florets of *Cichorium intybus*. Phytochemistry, 1969; 8: 1401–1403.
20. Weiss MR. Floral colour changes as cues for pollinators. Nature. 1991; 354:227-229.
21. Porat R, Borochov A, Halevy AH, O-Neill SD. Pollination induced senescence of Phalaenopsis petals. The wilting process, ethylene production and sensitivity to ethylene. Plant Growth Regulation. 1994; 15(2):129-136.
22. Ketsa S, Rugkong A. Senescence of *Dendrobium* 'Pompadour' flowers following pollination. Journal of Horticulure Science and Biotechnology, 1999; 74: 608-613.
23. Gard DL. y-tubulin is asymmetrically distributed in the cortex of Xenopus oocytes. Developmental Biology. 1994; 161:131-140.
24. Lovell PH, Lovell PJ, Nicholls R. The importance of the stigma in flower senescence in Petunia (*Petunia hybrida*). Annals of Botany. 1987; 60:49-59.
25. Zhang XS, O' Neill SD. Ovary and gametophyte development are coordinately regulated following pollination by auxin and ethylene. Plant Cell. 1993; 5:403-418.
26. Mo Y, Nagel C, Taylor LP. Biochemical complementation of chalcone synthase mutants defines a role for flavonols in functional pollen. Proceeding of National Academy of Science, l/SA. 1992; 89:7213-7217.
27. Vogt T, Poliak P, Tarlyn N, Taylor LP. Pollination-or wound-induced kaempferol accumulaton in Petunia stigmas enhances seed production. Plant Cell. 1994; 6:11-23.
28. Strauss MS, Arditi J. Post-pollination phenomena in orchid flowers. XII. Effects of pollination, emasculation and auxin treatment on flowers of *Cattleya* Porcia 'Cannizaro' and the rostellum of *Phalaenopsis*. Botanical Gazette. 1984; 143:43-49.
29. Duncan RE, Curtis JT. Intermittent growth of fruits of Phalaenopsis. A correlation of the growth phases of an orchid fruits with internal development. Bulletin of Torrey Botanical Club. 1942a; 69:167- 183.
30. Duncan RE, Curtis JT. Intermittent growth of fruits of Cypripedium and Paphiopedilum. A correlation of the growth phases of orchid fruits with their internal development. Bulletin of Torrey Botanical Club. 1942b; 69:353-359.
31. Woltering EJ, de Vrije T, Harren F, Hoekstra FA. Pollination and stigma wounding: same response, different signals. Journal of Experimental Botany. 1997; 48:1027-1033.