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Research Article

Pollination Leads to Oxidative Stress: An Analysis in the Floral Organs of *Coelogyne Cristata* Lindl. (Orchidaceae)

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ABSTRACT

Pollination, leads to early initiation of wilting in floral organs as compared to un-pollinated one, has been experimented in *Coelogyne cristata* Lindl. The senescence occurred within 7 days after pollination (DAP) which otherwise remained fresh for 18-20 days, without pollination; wilting in the floral organs viz. perianth, lip, column and ovary, initiated within 4-5 DAP (Stage-1 i.e.S1), progressed (Stage-2 i.e. S2) at 7-8 DAP and became a blackish crumpled mass in another 7-8 days and persisted as such thereafter. The onset of senescence has been analyzed by measuring oxidative stress evoked in the floral organs of *Coelogyne cristata*, measured initially in term of elevated electrolyte leakage in all the floral organs of pollinated flowers in comparison to unpollinated one. Other stress indicators including malondialdehyde (MDA) and H_2O_2, have also been observed to increase in their content while Ascorbic acid, an anti-oxidant, on the other hand, showed a decreasing trend. Comparatively, Lip and perianth experienced maximum injury; further the much damage was reported at advanced stage of senescence *i.e.*, stage-2. An application of inhibitors of auxin, namely TIBA (tri-iodo-benjoic acid; 0.25uM), and ethylene namely silver nitrate (0.25uM) to pollinated flowers, partially suppressed the oxidative stress and consequently delayed senescence suggesting thereby the involvement of these hormones in governing these changes in orchid flowers. Comparatively, $AgNO_3$ was more effective than TIBA in delaying senescence.

Key Words: Pollination, Senescence, Inhibitors, Orchids, Stress, Antioxidant, Floral organs

INTRODUCTION

Orchids, known for their intricately fabricated flower structure, commanded high place in the international market primarily due to long shelf life of their floral organs. The majority of flower have long shelf life in unpollinated state and perished quite early when get pollinated (Attri, *et al*. 2007, 2008; O'Neill, 1997). Role of auxins and ethylene, in controlling the pollination induced response, is well established (Attri, *et al*, 2007, 2008; Burg and Dijkman, 1967). It has been reported that pollen interaction with stigma either releases auxin that stimulates ethylene generation or –ACC; a precursor of ethylene (O'Neill *et al.*, 1993), which traverse across the flower organs leading to onset of their senescence by affecting metabolic events (Bui and O'Neill, 1998; O'Neill *et al.*, 1993). 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 colours (O' Neill *et al.*, 1993). Presently, a study was designed to investigate the fate of some stress related antioxidants (electrolyte leakage, malondialdehyde, hydrogen peroxide, ascorbic acids) in floral organs i.e. column, lip, perianth and ovary during senescence in pollinated flowers. The analysis was also performed in unpollinated flowers for comparative study. Flowers were also treated with TIBA i.e. tri-iodoacetic acid (polar transport auxin inhibitor) and silver nitrate (ethylene inhibitor) to probe the possible role of these hormones on floral senescence.

The overall aim of the study was:

(a) to examine the status of oxidative stress evoked during pollination
(b) manipulate the senescence related oxidative changes using inhibitors of senescence causing hormones i.e. TIBA; inhibitor of Auxins and Silver nitrates; inhibitor of Ethylene
(c) to develop an appropriate system for enhancing the floral longevity, since it was expected to add further to the floricultural significance of the orchids.

MATERIALS AND METHODS

The taxa under study *Coelogyne cristata* Lindl. was collected from its natural habitat i.e. Agra Khal (Garhwal region) and maintained in the 'Orchid house', Department of Botany, Panjab University, Chandigarh. The flowers were hand pollinated in the morning (9.00 a.m) and observed for visual changes daily. These were harvested at stage-1 (when first sign of wilting appeared)

after pollination (Fig. 1C) and at stage-2 (when the wilting was in progress) after pollination (Fig. 1D). The harvested flowers were dissected into perianth, lip, column and ovary. These components of the flowers were analyzed for peroxide, and ascorbic acid according to the following details.

Electrolyte leakage was calculated with the methods given by Lutts *et al.*, (1996) and defined as follows: EL (%) = $(L_1/L_2) \times 100$.

Where L_1= Initial conductivity

L_2= Final conductivity

Lipid Peroxidation was measured in terms of malondialdehyde (MDA) content described by Dhindsa *et al*., (1981).

Hydrogen Peroxide (H_2O_2) was estimated with titanium reagent (Teranishi *et al.*, 1974).

Ascorbic acid was analysed according to the method of Mukherjee and Choudhri (1983) using dinitropheylhydrazine (DNPH).

Treatment of Flowers with Inhibitors of Auxin and Ethylene

The hand pollinated flowers were sprayed with the inhibitor of auxin like triiodobenzoic acid (TIBA) and ethylene such as silver nitrate to restrict the endogenous effects of these hormones in flowers after pollination. The solution of these inhibitors was prepared by dissolving TIBA (0.25µm) and silver nitrate (0.25µm) in distilled water and sprayed on the flowers along with tween20 as a surfactant.

The data was analyzed statistically for standard error and ANOVA test using SPSS software. The variations in the parameters were found to be statistically significant and thus were not likely due to chance ($p < .01$).

RESULTS AND DISCUSSION

A comparative observation was made in *Coelogyne cristata* (Fig. 1A), before and after pollination and has been compiled in table 1.

As reported earlier in *Phalaenopsis*, *Cymbidium* species by Arditti (1992), *Aerides multiflora*, *Rhynchostylis retusa*, and *Cymbidium pendulum and C. aloifolium* by Attri *et al*. (2007, 2008), the floral development is affected by pollination. It is observed that unpollinated flowers of *Coelogyne cristata* (Fig. 1A) stayed fresh for 18-20 day while it took 8 days after pollination (Fig. 1D) to show senescence, suggesting thereby a rapid progression of senescence related events triggered by the process of pollination. Variation in colour of floral parts during regular record after pollination is again indicating a diverse biochemical mechanism that appears to be operative in different flowers. We have planned our study based upon the critical analysis of oxidative damage experienced by different floral organs (Perianth, column, lip and ovary; Fig. 1B), at two stages. First stage (Fig. 1C) was when the initiation of perianth wilting (S1) began and it was recorded at 4-5 days after pollination (DAP) while second stage (Fig. 1D) corresponds to complete wilting of perianth (S-2) that happened at 7-8 DAP. At the second stage, the perianth started showing signs of shrinking.

Table1. **Some visual changes in *Coelogyne cristata* Lindl. with respect to time.**

S. No	Events	Time taken
1.	Senescence in unpollinated flowers	18-20 days
2.	Senescence in pollinated flowers	7-8 days after pollination (DAP)
4.	Change in lip colour	Within 24 hours after pollination
5.	Increase in the size of column	1 days after pollination (DAP)
6.	Increase in the diameter of the ovary	1 days after pollination (DAP)
7.	Initiation of perianth wilting (Stage-I)	4-5 days after pollination (DAP)
9.	Perianth wilting progressed (Stage-II) i.e. just before shrinkage	7-8 days after pollination (DAP)

Figure 1. Morphological changes before and after pollination in *Coelogyne cristata* (A-D). *Coelogyne cristata* (Lindl.). A, Morphology of plant; B, Unpollinated flower showing different floral organs; C, Flowers showing initiation of senescence i.e. stage-1 at 4-5 DAP; D, Flowers with progressed senescence i.e. stage-2 at 7-8 DAP.

Further deterioration due to oxidative evocation was observed to enhance in the floral organs from S1 to S2. But this varied among different organs as in case of electrolyte leakage, an indicator of damage to membranes of the cells which increased markedly from first stage to the second one generally (Fig. 2). Comparatively lip and perianth showed higher membrane damage. This might explain the earliest visible signs of wilting and crumpling in these organs after pollination. The pollinated flowers of in present studies showed marked elevation of electrolyte leakage in all the organs, as observed by earlier authors in *Petunia* (Xu and Hanson, 2000. Ketsa and Rugkong (1999), on the other hand, recorded contrasting data, where pollination did not alter petal and sepal ion leakage in lip. The oxidative stress evoked during the floral senescence was blame to disturb the cell membrane properties, which in turn may primarily be responsible for losses of membrane phospholipids, increase in neutral lipids, increase in sterol to phospholipid ratio, and increase in the saturation: unsaturation index of fatty acids. It was reorted that the membrane polyunsaturated fatty acids are prone to oxidation either by enzymatic means [lipoxygenase (lox)] or through autoxidative events i.e. non-enzyme catalyzed (Fobel *et al*., 1987; Lesham, 1992; Thompson *et al*., 1998). Moreover, other

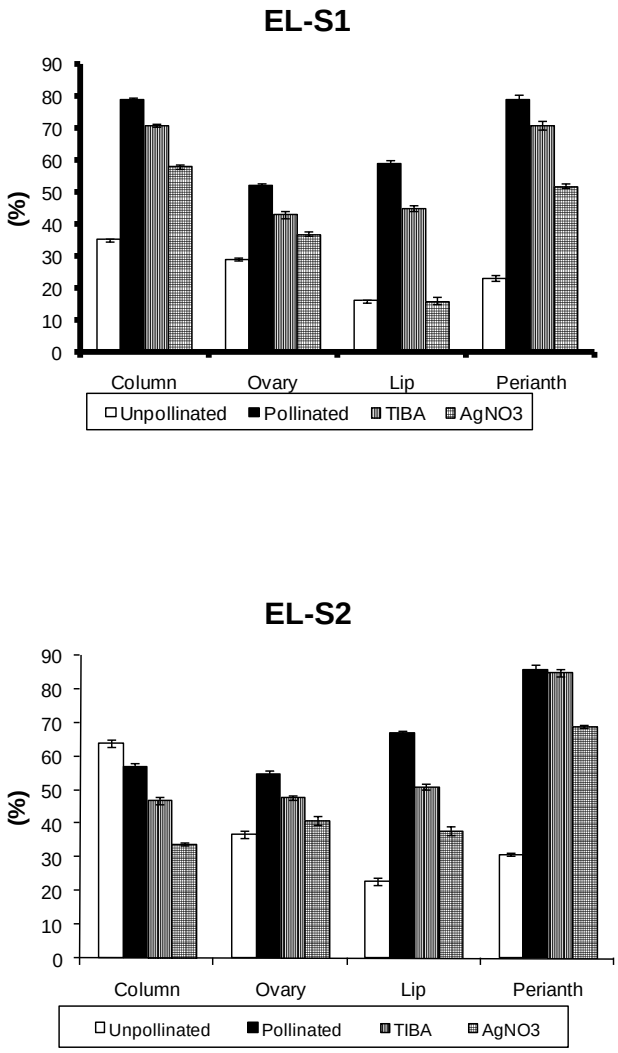


Figure 2. Electrolyte leakage (%) in different organs of *Ceologyne cristata* flowers at first stage (S1) and second stage (S2) after pollination.

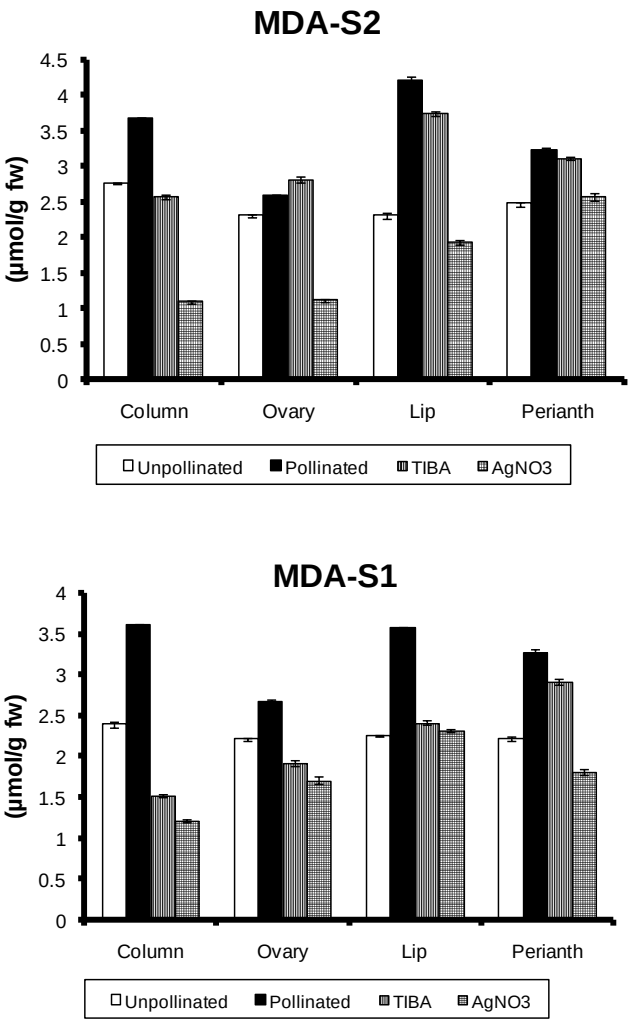
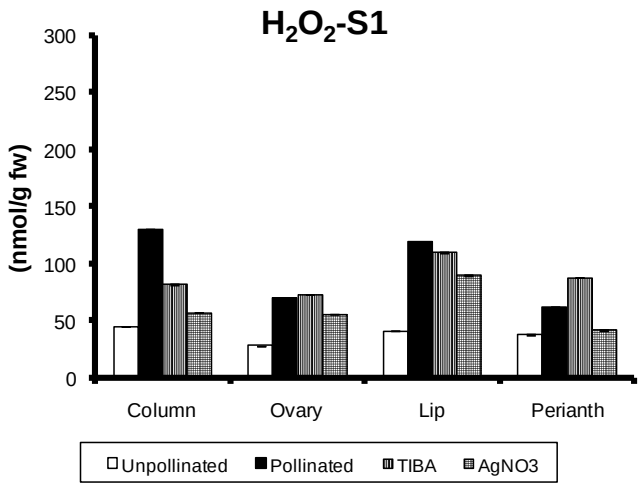
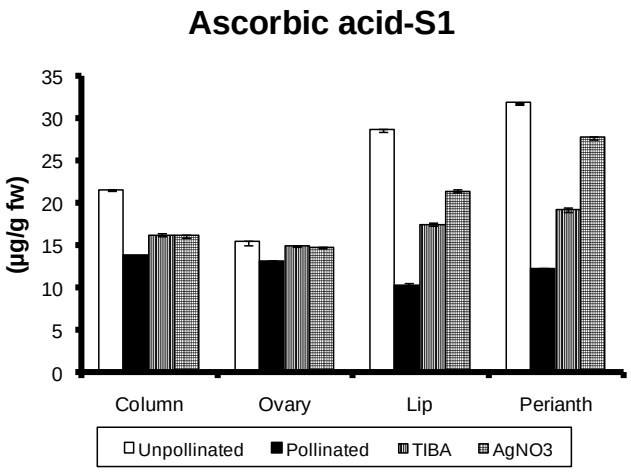
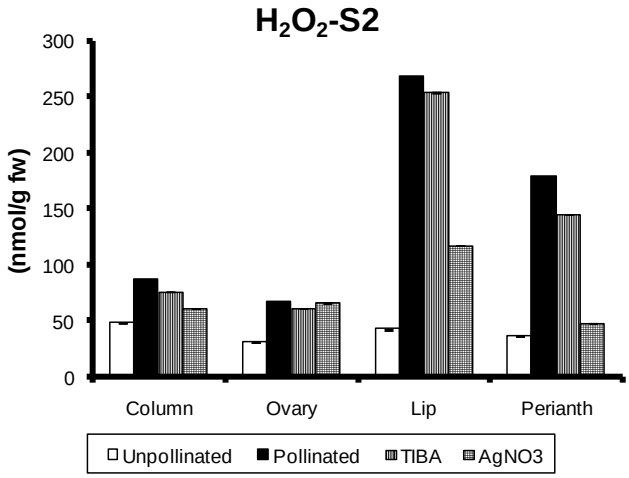


Figure 3. Malondialdehyde (umol/g fw) in different organs of *Ceologyne cristata* flowers at first stage (S1) and second stage (S2) after pollination.







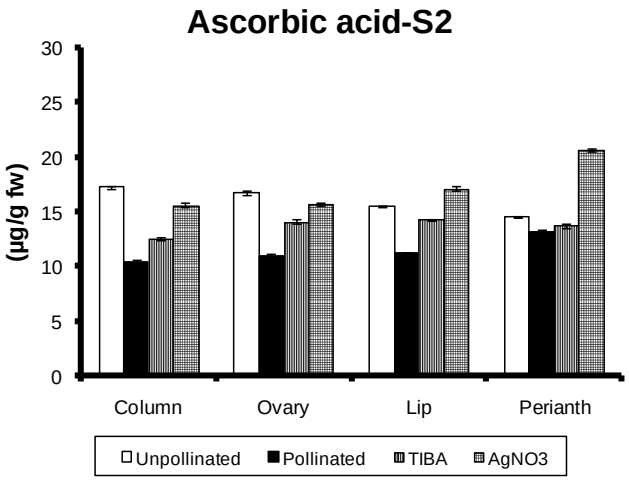


Figure 4. Hydrogen peroxide (nmol/g fw) in different organs of *Ceologyne cristata* flowers at first stage (S1) and second stage (S2) after pollination.

Figure 5. Ascorbic acids (umol/g fw) in different organs of *Ceologyne cristata* flowers at first stage (S1) and second stage (S2) after pollination.

factors like lipid peroxidation, estimated as thiobarbituric acid reactive substances (TBARS), accompanies the increase in lox activity and the products of peroxidation, are considered to perturb membrane function, partially, by causing increased membrane rigidification (Thompson *et al.,* 1998). In the present study, we estimated malondialdehyde (MDA), a product of lipid peroxidation, which elevated considerably in senescing tissues of pollinated flowers relative to the unpollinated ones (Fig. 3). Simultaneously, hydrogen peroxide (H_2O_2) also elevated markedly in pollinated flowers (Fig. 4) as compared to unpollinated ones that evidently suggested evocation of oxidative damage during senescence, the study had also been corroborated by Lin and Kao (1998). H_2O_2, choosen for the present study, is an active oxygen species and can react with superoxide radicals to form more reactive hydroxyl radicals in the presence of trace amounts of iron (Fe) or copper (Cu) (Thompson *et al*., 1987). The hydroxyl radicals, in turn, initiate self-propagating reactions leading to peroxidation of membrane lipids and destruction of proteins (Asada and Takahashi 1987; Bowler *et al*. 1992; Halliwell 1987). Thus, accelerated senescence in pollinated flowers could be attributed to H_2O_2-mediated increase in rates of oxidative reactions. On the contrary, ascorbic acid, a non-enzymatic antioxidant and free radical scavenger, showed lower levels in pollinated flowers compared to the unpollinated ones (Fig. 5). Ascorbic acid, an anti-oxidant molecule, acts as a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide. Additionally, it also acts directly to neutralize superoxide radicals, singlet oxygen or superoxide and as a secondary anti-oxidant during reductive recycling of the oxidized form of α-tocopherol, another lipophilic anti-oxidant molecule (Noctor and Foyer, 1998). Interestingly, reduced levels of ascorbic acid in pollinated flowers, undergoing senescence, indicates overriding influence of oxidative species associated with inducing the degeneration of tissues. Ascorbic acid was found to decline during senescence (Prochazkova *et al*., 2001).

An application of TIBA and silver nitrate were recorded to antagonise the effects of auxin and silver nitrate and partially suppressed the pollination-induced changes as reported previously (Attri *et al*., 2007, 2008; Jones and Woodson, 1997; Ketsa *et al*., 2000; Ketsa *et al*., 2006; Van Doorn, 1997; Zhang and O' Neill, 1993). Presently, we have successfully delayed floral senescence with the application of these inhibitors. TIBA treated flowers showed a delay in senescence related changes by 4-5 days while silver nitrate treated flowers delayed the senescence by 7-8 days. Thus, the present findings suggested that oxidative stress has a vital role in affecting the pollination-induced events and may be a key determinant in varying senescence duration in different orchid species. Ethylene compared to auxin appears to have a larger role in controlling the oxidative response. Future studies using more inhibitors having broad range of concentrations to extend the floral life span have been suggested.

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