Scanning Electron Microscopic Studies on Viscin threads of Pollinia of two Temperate Terrestrial Orchids

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Abstract
The viscin threads hold the pollens in various Pollen Dispersal Units (PDUs) in different orchids. Besides adhesion, they have immense significance in the life history of an orchid. The study envisages their importance and associated structural features for the pollen units in two north temperate orchid Eulophia dabia (D. Don) Hochr. and Zeuxine strateumatica(Lindl.) Schltr. based on the Scanning Electron Microscopic (SEM) studies.

Keywords: Viscin threads, pollinia, massula.

Introduction

The family Orchidaceae to which orchids belong have over 800 genera[5] and with species numbered as 24,500[8] and 25,316[16] is the largest family of angiosperms. This accounts for 8-10% of all the flowering plant species and one third of all the monocotyledons[1].They are found in a habitat ranging in conditions from arctic to tropical. There is profound diversification in their morphologies and genotypes and still they are in an active evolutionary flux. Known for their beautiful and long-lasting flowers orchids account for export/import trade of whole plants that exceeds $150 million and cut flowers of around $128 millions in the year 2000[17]. Unfortunately, being the victim of their own beauty, orchids are indiscriminately collected and whole orchidaceae finds mention in Appendix II of CITES and requires immediate attention for conservation and propagation. The present studies can significantly contribute to the taxonomy and conservation of the studied taxa.

The pollen diversity in orchids was the basis of classification of Orchidaceae and it reflects the importance of these structures in the life history of these beautiful group of plants and play a very vital and specialized role in the pollination mechanism[9]. The genetic modifications/adaptations of the sex cells are contributory factors responsible for the huge diversities in this group and it is influenced by the behavior of both male and female sex cells with respect to each other like: compatibility reactions and pollen stimulus on stigmatic surface for ovule development.

Especially the male partner (gametophyte) is quite interesting, as it exhibits huge structural variations, may be because prior to the fertilization it is the male counterpart that encounters the adversities of the environment and also its dependence for an appropriate pollinator for dispersal leading to pollination. Thus, the pollens are organized variously as monads, tetrads, massulae or pollinia in orchids. As many as eight types of pollen organizations in the form of pollen dispersal units have been recognized[20].The various forms followed an evolutionary sequence in Orchidaceae as: Monads- Tetrads- Massulae- Pollinia[11,15] representing simple form of organization to more complex forms.

Interestingly, such structural complexities are on account of a very specialized gluing material that bind pollens together. This viscious material is secreted by the tapetal cells of anthers- called as viscin and develop to form thread like structures- the viscin threads[3]. There are little reports on these structures as well as their functional significance for the male gametophyte[2]. Do they serve as templates to organize the male gametophytes or are an evolutionary adaptations or mere structural vestiges!.

It is with this view that an attempt has been made to quantify the ecological and adaptive consequences of these viscin threads in two orchid species *Eulophia dabia* (D. Don) Hochr. (Fig.A,B) and *Zeuxine strateumatica* (Lindl.) Schltr. (Fig.C,D) based on scanning electron microscopic studies of their pollen units.

Material and Methods

The pollen units were collected in dry vials (Borosil) from two orchid species *Eulophia dabia* and *Zeuxine strateumatica* of which the former is found growing among Sachharum bushes (Fig.A) at village Tira and Dhanas nullah in the outskirts of Chandigarh region while latter is from open grass lands (Fig.C) of the P.N. Mehra Botanical Garden, Panjab University, Chandigarh and Dhanas Nullah. The collected plants were identified using the herbarium specimens preserved in the herbarium of Botany depertment, Punjab University, Chandigarh as well as flora of North-West Himalayas[6]. The vouchers were preserved in 70% ethanol and submitted in the herbarium of Botany department, Punjab University, Chandigarh, India.

For SEM studies, the pollen units of both species were unacetolysed; transferred to absolute alcohol and then separately mounted on double adhesive tape on a metallic stub, followed by coating with palladium-gold[22]. The specimens were examined under Scanning electron microscope (JEOL.JSM.1600).

Pollen viability was tested by TTC assay test. The TTC was prepared as follows: 0.5% solution 2,3,5- Triphenyl tetrazolium chloride in 1% of sucrose solution at pH 7.2. and is kept in dark bottle after preparation until use. For testing

viability, the pollinia were transferred in a vial containing the solution and incubated at 35ºC for 3hrs. The viable pollens stained red and weak to nil coloration for partial and non-viable ones respectively.

Results and Discussion

Both the species undertaken for present study belong to highly advanced monandrous forms in Orchidaceae and have pollen units as pollinia in *E. dabia* (subfamily: Epidendroideae) and massula in *Z. strateumatica* (subfamily: Orchidoideae). In both these taxa the basic pollen subunit is tetrad.

In *E. dabia*, pollinia are indistinctively sactile and hollow structures (Fig. E) similar to orchids *Arethusa* and *Calopogon* of subfamily Epidendroideae[10,19] and the massulae appeared overlapping or superposed upon each other, another trait that is useful in defining a taxonomic group[7]. Additionally, the pollinia is smooth, hard, fine and gyrate (Fig. F). The pollens were inaperturate in confirmation with earlier reports for most of the orchid pollen[22].

A number of massulae are observed to unite together to form a sactile pollinia in *Z. strateumatica* similar in lines with earlier studies[14] (Fig. G). The masullae are pyriform in shape, triangular in outline and are positioned adjacent in locules or juxtaposed. The texture of each massula is reticulate exine (tectate) with lobed and branched muri that are heterobrochate (Fig. H). The pollens were inaperturate.

The pollinia appear to be calymmate i.e. exine is not deposited on internal pollen grains in *E. dabia* leading them to be more compact and hard structures while in *Z. strateumatica* the pollen tetrads were calymmate while masullae were acalymmate and thus appear to be more friable. In subtribe Disinae pollen tetrads are calymmate, but the massulae and pollinia are acalymmate[4]. The pollens as they undergo cohesion in a pollinia must be resisted for germination to some extent due to the interference caused by pollen walls[12]. The absence of exine in inner pollens as observed in *E. dabia* appears to be an adptation (or evolutionary compromise!) that helps alleviate this interference.

Unlike other monandrous orchids as observed in *Eulophia hormusjii* and *Pachystoma senile*,[10] no dense granular sporopollenin like deposits were present in these monandrous species. If any granular substances were seen they were believed to be the remains of viscin, the strong acetolysis resistant substance.

In *E. dabia*, the pollen masses have thin margins of the pollen walls in the region of elastoviscin threads joining the segments of the pollinia then at general surface (Fig. I), the reason is unknown but could have evolutionary consequence. The elasoviscin threads show considerable variation in shape and size[10] and in the present studies they appeared to be of two kinds: inter-segmental and intra-segmental. They appear to arise from the outer exine but whether intine is also involved in their formation is to be confirmed by Transmission Electron Microscopy (TEM). The former are non-uniform in thickness (Fig. J), elongate and glutinate the segments together and probably serve to perform cytomixis. The later are more uniform, bind the pollens in a mass and also contribute to the ornamentation of the pollinia (Fig. K).

However, in *Z. strateumatica* they even serve to attach the massulae with the stipe of the pollinarium (Fig. H). There is no caudicle in *Z. strateumatica*[24] and it suggesting that these play direct role in binding massula to the pollinator. Both kinds of the above mentioned threads remain as cytomictic channels even after the dissolution of callose, as they persist till the pollinia maturity (Fig. L).

The elastoviscin threads exhibit variabilities at both intergeneric and intrageneric level. Thus they appeared presently as highly branched in *E. dabia* that connect the meiocytes of the two segments while in *Z. strateumatica* they appear to be less branched and earlier in *Habenaria bractescens* and *H. secunda* these observed to be branched and in *H. gourlieana and H. paucifolia* as unbranched[21]. They appear to bridge together the same information shared by the two neighbor meiocytes with the meiocytes of the adjacent segment (Fig. M). The cytomictic channels forming syncytium, as observed in *Z. strateumatica*[25] speak of meiotic synchrony within the massulae but in *E. dabia* besides meiotic synchrony, the role played by these channels between the meiocytes of adjacent segments appears interesting and can further be explored by transmission electron microscopy (TEM) studies.

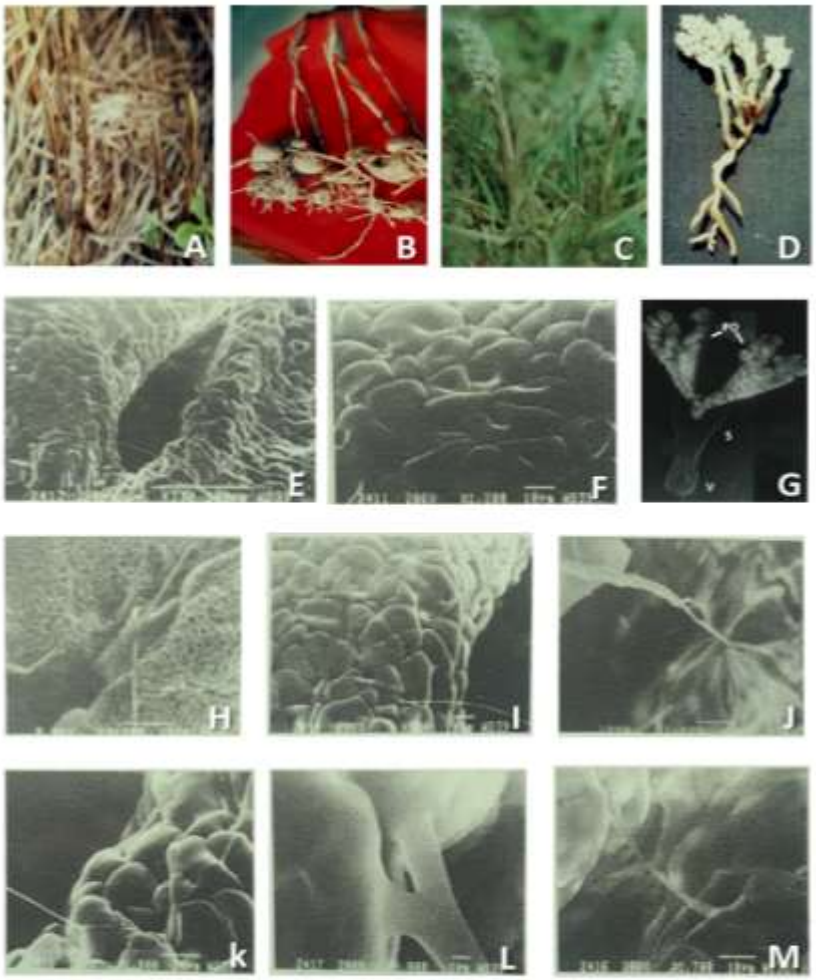
It is no doubt that these viscin threads glutinate the meiocytes and maintain harmony during mitosis subsequent to meiosis, they even maintain the final size and shape of pollinarium. On the stigmatic surface they contribute to the compatability reactions of the male gametophyte and pollinia stimulus for development of female gametophyte following pollination. As the season proceeds towards more warm conditions and low humidity, they eventually undergo degeneration either by breaking or withering after dryness. Whether their eventual loss after pollinia maturity is a primitive/advanced feature or is in response to seasonal variability is a big question and requires more elaborate expeditions. There is eventual loss of pollen viability, 3hrs after these channels have degenerated.

These threads thus appear to enhance the survival value of the pollinia, by providing them nutrients and storage of additional moisture that tend to mediate harmomegathic effect[12] (xeric adaptation for survival of pollens). The main purpose of the harmomegathic effect is to protect the male gametophyte during pollen presentation and dispersal, wherein the infolding of pollen wall due to water loss/gain

tends to accommodate the changes in the cytoplasmic osmotic pressures.

The functional implication of viscin threads in the pollen units of the studied taxa suggests that pollen to ovule ratio is exacting as the pollen loss cannot be compromised during transit and to avoid minimal wastage, pollens are held together as a single unit. These threads help in swelling of the pollinia on the stigmatic surface by conducting the moisture from them and organizing them in spatially most significant orientation so that more pollen grains can came in contact with stigmatic surface. It is necessary event since maximum number of pollens has to germination to affect higher rates of fertilization.

As mentioned earlier, the Transmission Electron microscope studies (TEM) are of immense helpful in further studies. However, the connecting channels owing to be very fragile get destroyed during the sample preparation for TEM and freeze-fracture technique is suggested for sample preparation. Finally, besides realizing the significance of viscin threads, a more comprehensive understanding of other mechanisms involved can be expected by comparative studies of other taxa.



Legends to Figures: Fig.A. *Eulophia dabia* in natural habitat among Saccharum bushes, Fig.B. *Eulophia dabia* collected specimens prior to their preservation, Fig.C. *Zeuxine strateumatica* stands in open grassy lands, Fig.D. *Zeuxine strateumatica* habit of a plant, Fig.E. Hollow pollinia and persistent cytomictic channels in *Eulophia dabia,* Fig.F. The gyrate and smooth surfaced pollens in *Eulophia dabia* pollinia, Fig.G. Sactile pollinium of *Zeuxine strateumatica,* (PO-pollinia; S-stipe; V-viscidium), Fig.H. Pollinia of *Zeuxine strateumatica* with reticulate exine and branched muri also visible is the stipe attached to the massulae with thier connecting channels, Fig.I The thin contact surface walls and elasoviscin thread joining the pollinia segments in *Eulophia dabia,* Fig.J. The inter-segmental elstoviscin threads elongate with non-uniform thickness, Fig.K.Intra-segmental threads,short and stout contributing to pollinia surface patterns, Fig.L. Two meiocytes of a segment as they are connected, Fig.M. Information sharing channels

Conclusion

The observed elastoviscin threads have contributed to a variation in the shape and sizes of the pollens in orchidaceae and therefore have significantly played a role in the taxonomy of the family. The SEM examinations reveal that their structural intricacies may have profound functional significance and reflect the evolutionary landmarks achieved by these group of plants. These threads are helpful in preserving the pollen and maintaining it prior to its germination, preventing its dehydration, helpful in efficiently delivering it to the stigma by the pollinator, orienting pollinia on stigmatic surface (threads may be contractile) and as conducting channels.

Since in orchids, the seeds are non-endospermic with highly reduced embryo, it suggests that during ovule production, energy investment per ovule production is reduced and in turn is being utilized in production of more number of ovules. Now for an efficient fertilization of all of these ovules, equally large amount of pollens must be deposited on stigma surface. Thus, theses two factors 'small seeds' and 'need for efficient pollen export' interplayed to exert their selective pressures for evolution of pollinaria[13].

The elastoviscin threads hold pollens together and favoured a pollen packaging strategy that allowed removal of all of the pollen from the anther in one visit with minimal wastage in transit and minimal probability of it being deposited on foreign stigmas. Despite, such complexities of the pollen architecture in Orchidaceae and the intricate patterns developed therein act in a way to help allure its pollinators and mediate a specialized and successful pollen delivery mechanism. This together with huge number of species has kept orchids high on the evolutionary scale as among other monocotyledons. However, despite these specializations, orchids rendered unsuccessful to show their prominence in the ecosystems in the way the other generalist pollination species of Asteraceae and wind pollinated Poaceae are capable of.

Nevertheless, Orchidaceae appears to be in an active evolutionary flux and as we learn more about them we can conserve them in a better way and application of sophisticated technologies can be a boon to this approach[18]. The present investigations further suggest that such studies can be helpful in designing various breeding programmes, ascertaining phlogeny and preservation/storage of haploid germplasm of various orchid species.

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