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Recognition of pollen adhering threads in six members belonging to Onagraceae and Caesalpiniaceae and their ultrastructural observations

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ABSTRACT

The pollen connecting threads help in the formation of pollen aggregates ensuring entomophilous pollination *en masse* thereby reducing the anemophillous loss of pollens. The study involves the recognition of pollen adhering threads in four members of Onagraceae *viz. Ludwigia perennis, Ludwigia octovalvis, Ludwigia prostrata and Ludwigia adscendens* and the remaining two of Caesalpiniaceae, *viz. Amherstia nobilis* and *Caesalpinia pulcherrima.* Their micromorpholgical observations reveal that among the four members of Onagraceae, *Ludwigia prostrata* and *Ludwigia octovalvis* exhibits tetrad pollen grains where the viscin threads form connections between the tetrads thereby forming large pollen aggregates, while in case of *Ludwigia adscendens* and *Ludwigia perennis,* the viscin threads are smooth and intermingles with the pollen monads forming loose lumps of pollen grains. In Caesalpiniaceae, the slimy strands are short and branched, connecting the pollen grains as encountered in *Amherstia nobilis* and *Caesalpinia pulcherrima.* The presence of 'pollen connecting threads' in the species *Amherstia nobilis* has been reported in this paper for the very first time.

Key-words: Pollen connecting threads, anemophillous, pollination, en masse, Onagraceae, Caesalpiniaceae.

INTRODUCTION

'Pollen connecting threads', which are the striking feature of few angiosperm families, have remained a topic of interest from a very early date. The fundamental structure, origin and function of these threads have been studied and revised by various authors from time to time. Major works on pollen connecting threads have been carried out by Erdtman (1952), Hesse (1984b), Waha (1984), Patel (1985), Vogel (1988), Halbritter (1997) and Skavarla et al. (1975) to name a few of them. Pollen grains occur in clusters within the anther of angiosperms and the most common type of pollen clusters are the sticky pollen grains or polyads (Walker & Doyle 1975, Hesse 1980b). A different type of pollen cluster occurs by means of threads or fibres which are regarded as 'pollen connecting threads'. These are thread like strands which binds the neighbouring pollen grains together within the anther and play significant role in pollination ecology of the species. The pollen connecting threads helps in the formation of pollen aggregates which ensures pollination *en masse*, thereby reducing the loss of pollen grains by anemophily.

These threads are basically of two types based on the presence of sporopollenin (Hesse 1986a). 1). The sporopolleninous viscin threads which are resistant to acetolysis, develops from the ektexine thereby forming an integral part of the pollen wall (Skavarla et al. 1978, Nowicke et al. 1988). These threads are basically the

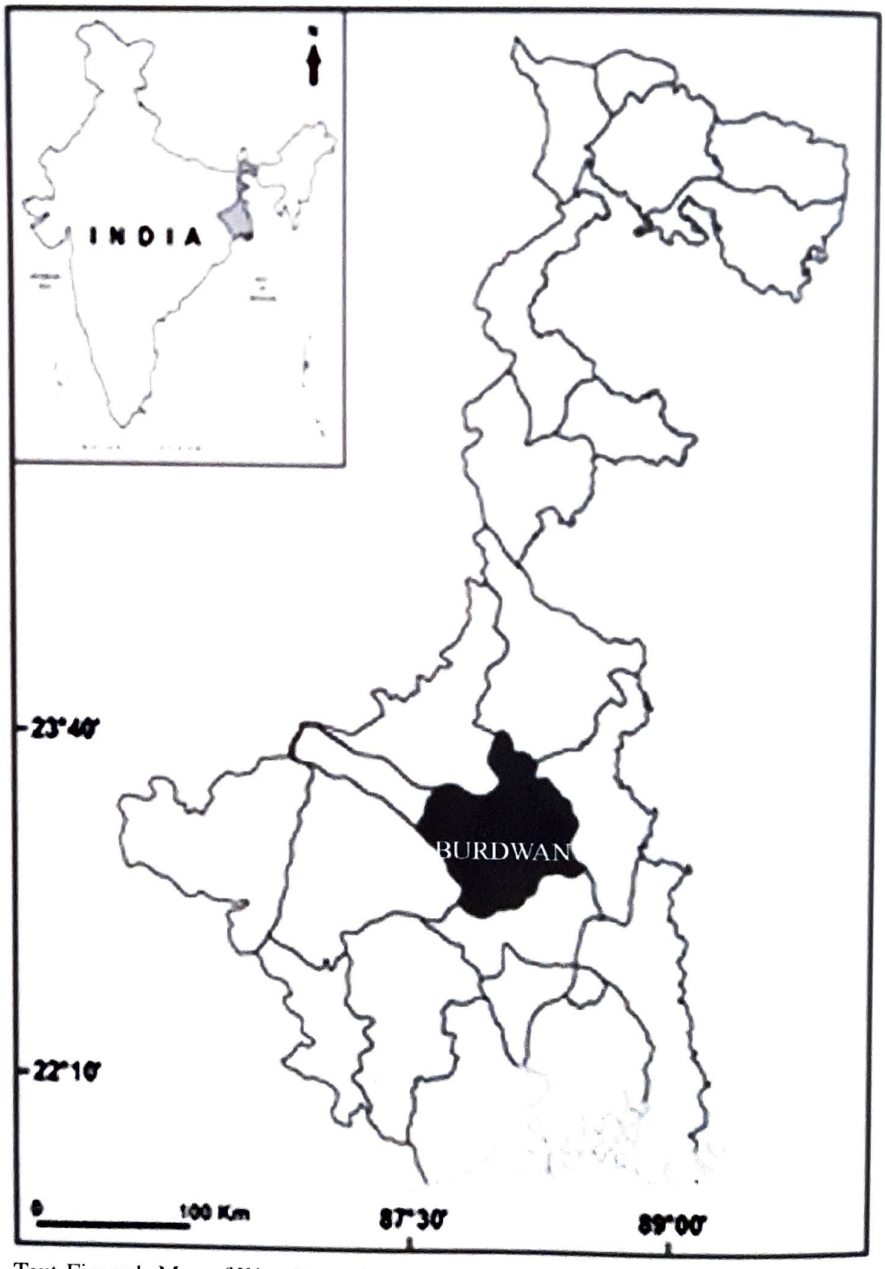
extensions of the ektexine (Hesse 1984b). They occur strictly among three unrelated angiosperm families Onagraceae, Ericaceae and a single member of Caesalpiniaceae (Hesse 1984b). On the basis of morphology the viscin threads may be smooth, compound or beaded as encountered in various species of these families. 2). The non sporopolleninous slimy strands which get dissolved during acetolysis are derived either from the cell or from cellular secretions (Hesse 1986). They are common in many angiosperm families including Caesalpiniaceae. Although these threads are of different chemical nature and structure but they share similar functions.

Ultrastructural studies of 'pollen connecting threads' of members of Onagraceae have been investigated by many workers in different parts of the world, namely in Japan by Ikuse & Sahashi (1984), in Northeast Anatolia by Makbul et al. (2008), in Alaska by Skavarla et al. (2008) and in Pakistan by Perween & Qaiser (2013).

There are no reports of study of ultrastructural studies of pollen connecting threads from India. The present study reveals the micromorphological details of viscin threads of four members of Onagraceae belonging to the genus *Ludwigia* and slimy strands of two members of Caesalpiniaceae belonging to the genus *Caesalpinia* and *Amherstia*. The presence of 'pollen connecting threads' in the species *Amherstia nobilis* has been reported in this paper for the very first time.

MATERIAL AND METHODS

The polleniferous material of a total of six species belonging to two angiosperm families, Onagraceae which includes *Ludwigia perennis*, *Ludwigia octovalvis*, *Ludwigia prostrata* and *Ludwigia adscendens* and that of Caesalpiniaceae which includes *Caesalpinia pulcherrima* and *Amherstia nobilis* were freshly collected from Burdwan [23.2333° N, 87.8667° E], West Bengal, India (Text-Figure 1) for the present study. The pollen grains were investigated using both light and scanning electron miocroscopy. At first the pollen grains of both the families were studied without any treatment. After that they were subjected to acetolysis and it was confirmed that the pollen connecting threads of the members of Caesalpiniaceae are not resistant to acetolysis. So, for the final study, the pollen grains of the members of Onagraceae were acetolysed according to Erdtman (1952) while that of



Text-Figure 1. Map of West Bengal showing the study area

Caesalpiniaceae were kept unacetolysed as they are not resistant to acetolysis. Pollen preparations of both the families were mounted in glycerine jelly on glass slides with 0 no. coverslip and were studied under Olympus GB microscope. The photographs were taken under a Leitz laborlux S (Germany) microscope with Leica DFC 295 digital camera attachment. For scanning electron microscopy, acetolysed pollen grains of Onagraceae were used whereas in case of Caesalpiniaceae members, freshly dehisced anthers were delicately touched on the metallic stub and coated with gold by an Eiko Engineering (Japan) Ion Coater and observed using a Hitachi (Japan) S-530 SEM aided with a digital photomicrographic system.

OBSERVATIONS AND DISCUSSION

Micromorphological details of the selected taxa of Onagraceae:-

Ludwigia prostrata Roxb. (Text-Figure 2A, B)**:**

Individual pollen grains are present in tetrads and these tetrads are entangled with each other by means of the viscin threads forming loose lumps. The viscin threads are long, slender and smooth being uniform in thickness. They arise from the area near the apertural margin and extend greatly connecting the other tetrads.

Ludwigia adscendens (L.) H. Hara. (Text-Figure 2C, D):

Individual pollen grains are found in monads which are intermingled with each other by the viscin threads. The viscin threads are smooth and slender. They arise from the margin of the aspidote pore of the pollen grains. The threads are coiled and twisted as they connect the nearby single pollen grains together forming large pollen aggregates.

Ludwigia octovalvis (Jacq.) P. H. Raven. (Text-Figure 2E, F):

Pollen grains are found in tetrads which bear tufts of viscin threads arising from their apertural margins. The threads are smooth and slender having uniform thickness. A number of pollen tetrads are connected with each other by the viscin threads.

Ludwigia perennis L. (Text-Figure 2G, H):

Pollen grains are found in monads which are entangled with each other by the viscin threads. The viscin threads are heavily deposited at proximal surface of the pollen grains. They are compound in structure. They consist of a group of smooth threads which are adhered together which gives them a somewhat thicker appearance.

Micromorphological details of the selected members of Caesalpiniaceae:-

Amherstia nobilis Wall. (Text-Figure 2I, J):

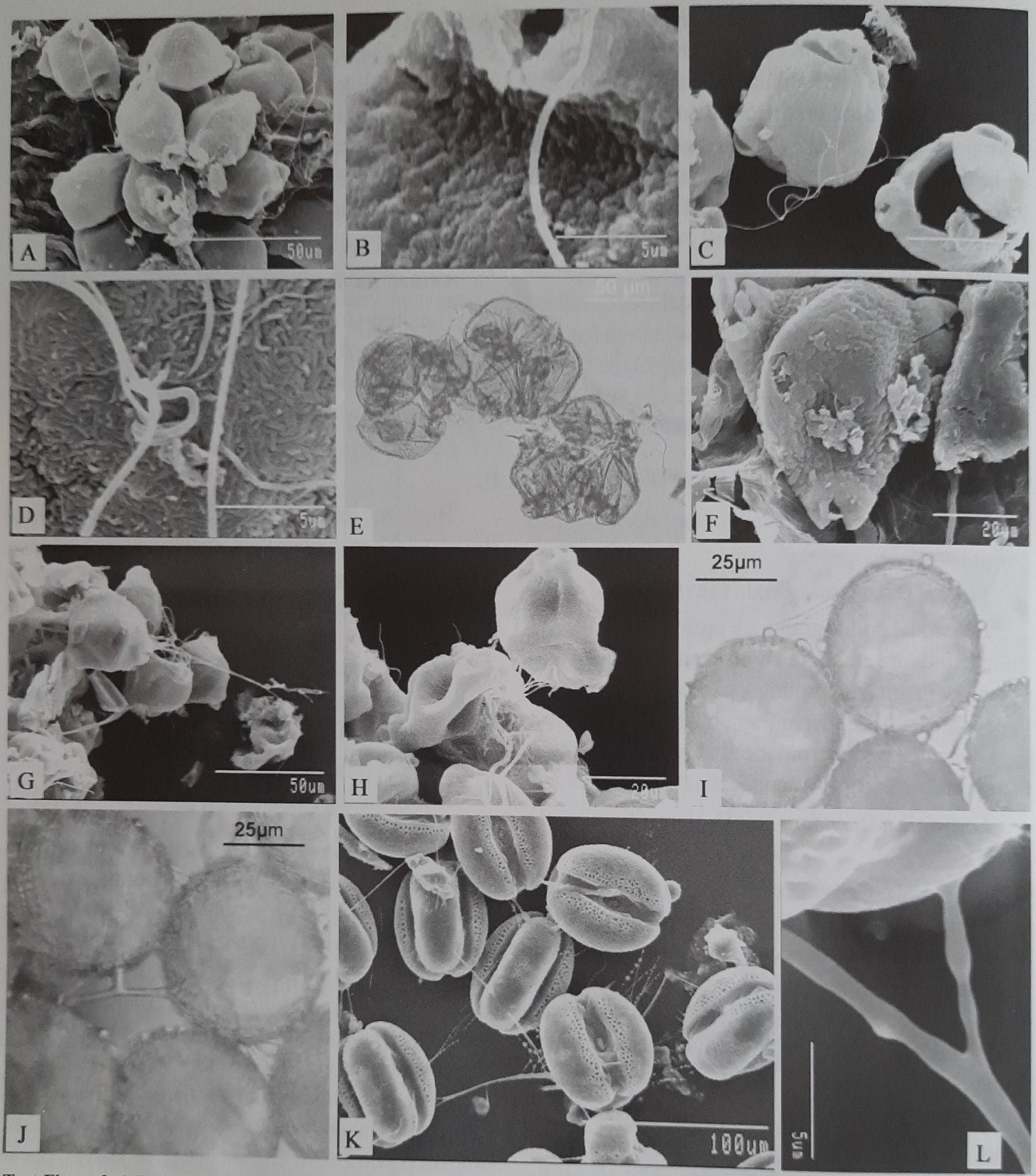
The slimy strands, connecting the pollen grains are very short, smooth and dichotomously branched having uniform thickness**.** The pollen grains are totally coated with pollenkitt which appear as droplets on the surface.

Caesalpinia pulcherrima L. (Text-Figure 2K, L):

The pollen grains are clustered together by the slimy strands which are longer, thin, smooth and slender. Two types of adhesion threads have been encountered in this species. One is smooth which is not uniform in thickness, having undulating surface and are dichotomously branched, while the other type is typically beaded. It has the appearance of a number of small droplets attached by a fine string. They are not forked and shorter in length.

CONCLUSION

The present work reveals that the entomophilous angiospermous taxa have adapted various devices to check the anemophilous pollen loss. The sporopolleninous viscin threads connect the neighbouring tetrads of pollen grains together as encountered in *Ludwigia octovalvis* and *Ludwigia prostrata*. These tetrads are entangled with each other forming large aggregate of pollen grains. In case of *Ludwigia adscendens* and *Ludwigia perennis* the viscin thread binds the monads together, forming loose lumps of pollen grains. The viscin threads tend to appear as tufts of hair emerging from the area beside the margin of the aperture i.e., from the aspid. The attachment points are always located near the proximal pole even when they are arranged in tetrads as mentioned earlier by Ikuse & Sahashi (1984). In all the above investigated species of Onagraceae the viscin threads are smooth and slender. Among the members of Caesalpiniaceae,



Text-Figure 2. A-B: *Ludwigia prostrata* (A) SEM photographs showing the pollen grains are in tetrads and few tetrads are clumped together forming a large pollen aggregate, (B) Single pollen grain showing a portion of the viscin thread. C-D: *Ludwigia adscendens*, (C) SEM *Ludwigia octovalvis*, (E) Aggregates of pollen tetrads connected by viscin threads. (F) A single grain with a portion of viscin thread. G-H: *Ludwigia perennis*. (G) Viscin threads are smooth and adhering to the neighbouring pollen grains. (H) Magnified view of the viscin thread. I-J: *Amherstia nobilis*: (I) Slimy strands forming pollen aggregates, (J) Slimy strands showing branches. K-L: *Caesalpinia pulcherrima*, (K) SEM photographs showing slender and smooth slimy strands together with the beaded strands adhering the pollen grains together, (L) Magnified view of the slender, smooth and branched slimy strand.

in *Amherstia nobilis*, the non-sporopolleninous slimy strands are slender and smooth. They are short and branched, thereby adjoining the neighbouring pollen grains. These slimy strands are most probably composed of pollenkitt which assumes a rope like structure (Halbritter & Hesse 2000). In case of *Caesalpinia pulcherrima* two types of pollen connecting threads have been recognised. One is smooth, slender, branched and comparatively longer in length while the other type is short and beaded. They are composed of lipids and cytoplasmic contents, both deriving from tapetal cells only as observed in all the investigated species of Caesalpiniaceae (Hesse et al. 2000).

Hence, the pollen connecting threads play vital role in pollination ecology of the species. Various authors have deduced the function of these threads in a number of ways. The primary function of viscin threads is to attach pollen at the border of the opened pollen sac thereby preventing them from falling off prematurely (Rowley 1987). The transfer of larger pollen association i.e., pollination *en masse* increases the chance of fertilization of ovule (Hesse et al. 2000) and prevents loss of pollen grains by anemophily. Pollen grains associated with pollen connecting threads represent a highly specialized mode of pollination.

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