Structure and Cytochemistry of the Stigma and Style of *Datura inoxia* Mill. (Solanaceae)

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Abstract
The pollen-pistil interaction involves the transfer of chemical signals from pollen to the stigma during the time of pollination. The structure of pollen grains and the stigmatic surface strongly supports the effective pollination. After the germination process the pollen tube travelled through the stylar tissue. The chemical substances in the stylar region direct the pollen tube for successful fertilization.
Investigations were carried on the structural anatomy and cytochemical staining particularly for presence of protein in the tissues of the stigma and style of *Datura inoxia* Mill. (Solanaceae). The stigma was found to be wet and contain surface proteins as revealed by the CBB stain. The style was long and solid with central core of transmitting tissue throughout the length of the pistil. The transmitting tissue is compact and oval shaped. The intercellular spaces through which the pollen tubes grow contain many proteinaceous substances. The present work indicated the location of proteins in the region of stigmatic surface and in the stylar tissue.

Keywords: cytochemistry, pollen-pistil interaction, transmitting tissue, pollination.

1. Introduction
In angiosperms the pistil tissue provides both physical and chemical support to the pollen and directional guidance to the pollen tube growth process. A series of interactions between the pollen and the stigma occurred during pollinations. The studies on the details of the pistil are very important aspect to understand the process of pollen-pistil interactions and obviously the sexual reproduction [1]. Extracellular proteins have been shown to be invariably present on the surface of the stigma where pollen grains are received and in the path of the pollen tube in the style [2, 3, 4]. The pistil has developed mechanisms to recognize only the compatible pollen and permit the growth of pollen tube to reach the female gamete.
To understand the role of proteins in the process of fertilization the present work deals with the structural and cytochemical aspects of the stigma and style of *Datura inoxia* at the time of pollination.

2. Materials and methods
Fully bloomed flowers of D. *inoxia* were collected from departmental garden at Amravati and used for the study. The pistils were excised from the flower.
The fresh stigmatic head was cut and softened in acetoalcohol in the ratio 1:1 and then immersed in 0.2% CBB stain solution for 5 minutes. The blue stained stigmatic head then destined for 20 minutes. The properly stained stigmatic head was placed on the slide and then macerated with slight pressure on the cover-slip and then the slide was photographed.
The pistils were fixed in FAA fixative and then the fixed material was subjected to the microtomy and the slides were photographed with the help of trinocular fluorescence microscope (Axiostar HBO 650/AC Carl/Zeiss).

3. Results
Stigma: The mature pistil has very long and flexible style (Fig. 1). The stigma was large, pale whitish and covered by a yellow colored cap (Fig. 2). The stigma was found to be wet and non-papillate (Fig. 3 and 4). The pollinated stigmatic tissue which has an irregular shaped thin walled parenchymatous tissue and a central core of compactly arranged cells. The tissue shown much non-germinated and germinated pollen grains (Fig. 5). The germinated pollen grains and the stigmatic tissue both were found to be positive to protein stain (Fig. 6).

Style: The style is very long, solid and straight. It has been observed that the style was quite flexible in pollinated flower. The semithin section revealed almost circular outline comprises of an outer cuticle, an epidermis layer, parenchymatous cortical tissue and a central transmitting tissue (Fig. 7). The epidermis was composed of a single layer of cells. Adjacent to epidermis were 8-10 layers of cortical cells. The two vascular bundles present at either sides of the central transmitting tissue revealed positive result for the CBB stain (Fig. 8).
The distinguish features of both epidermal and cortical cells was the presence of proteins in cell wall region and cytoplasm was clear with almost no staining (Fig. 9).
The cylindrical transmitting tissue was located in the centre of the style and was found to be continuous with the stigmatic tissue. These cells appeared rounded in transverse section and compactly arranged. These parenchymatous cells with intense staining signified that cytoplasm of the cells were localized with more proteins (Fig. 10).

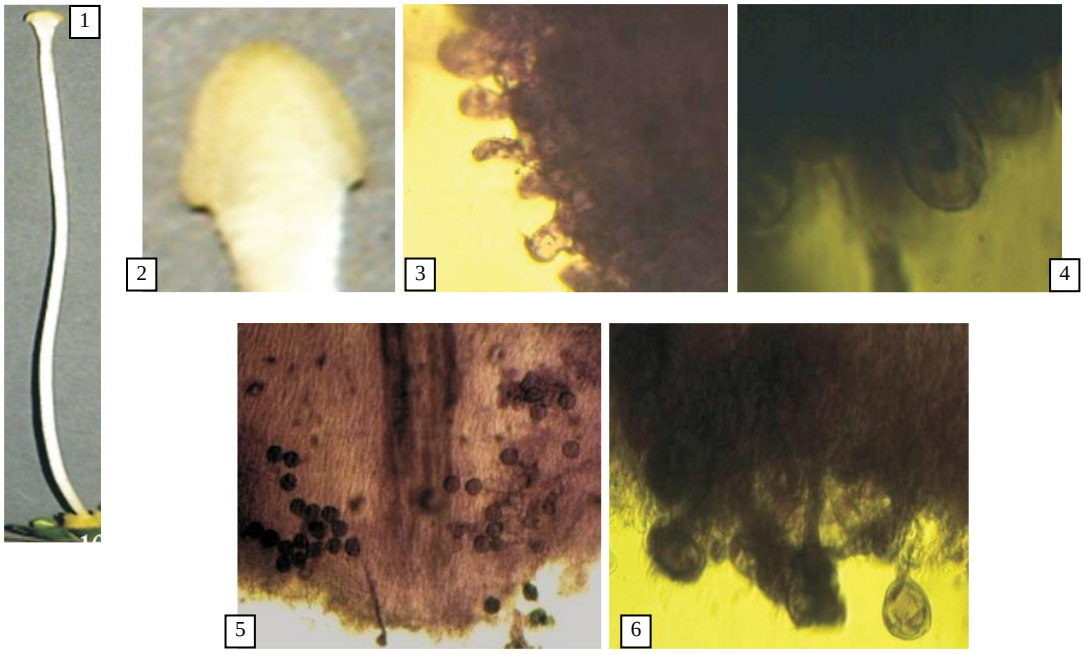

Fig 1: Morphology of pollinated pistil. **2:** Triangular Stigma showing yellow cap. **3-4:** The stigmatic surface showing the presence of proteins. **5:** The inner surface of stigma showing non-germinated and germinated pollen grains. **6:** The germinated pollen grain on the stigma showing CBB stain

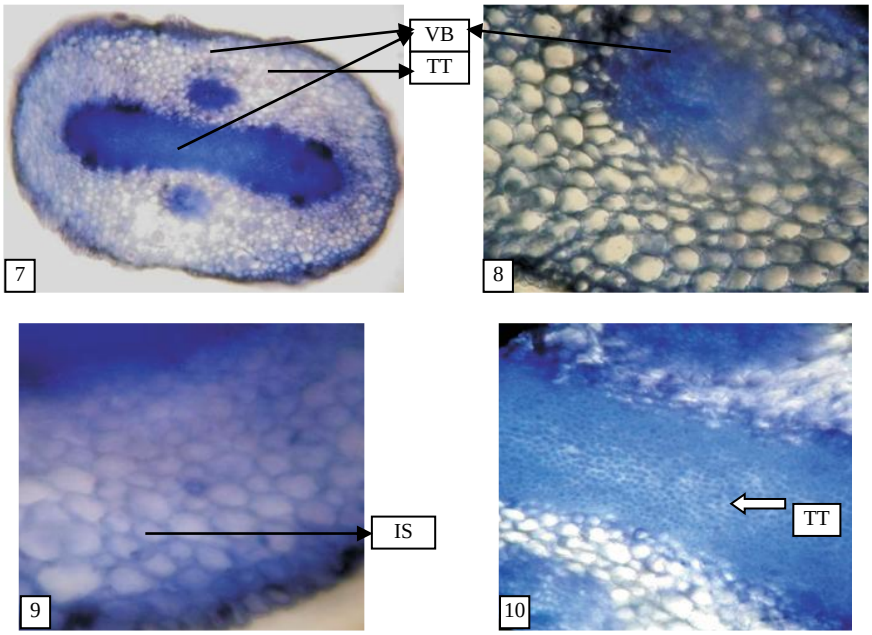

Fig 7: The transverse section of the style showing the TT, two VB. **8:** The cortical cells showing vacuoles. **9:** The cell wall and intercellular spaces (IS) showing intense blue stain. **10:** The compact transmitting tissue (TT) more positive to CBB stain.

4. Discussion

The stigma was found to be wet and non-papillate according to the classification by Heslop-Harrison and Shivanna [5]. The stigma is having a cap and appeared triangular revealed that shape of the stigma played a critical role in the early events when pollen reached the stigma by providing receptive surface [6]. The stigmatic surface shows positive reactivity to the basophilic CBB dye indicating proteinaceous nature. Similar was previously studied by Rejon *et al.* [7].

The style is solid having transmitting tissue in the centre, similarly the systems studied with solid styles are *Primula vulgaris* [8], *Olea europaea* [9], *Nicotiana sylvestris* [10] *Tibouchina semidecandra.* [11]

The transmitting tract staining indicated the presence of protein as one of the secretion product. This was investigated in many species. [9-10, 12-14]. The intense staining in the pistil is noticed after pollination this may be due to the release of protein from the pollen wall to the pistil after contact. This indicated the role of pollen and pistil proteins in the pollen-pistil interactions [15-16]. According to Heslop-Harrison *et al.*

[17] and Cresti *et al*. [18], the proteins present on the stigma and style are involved in the recognition of pollen during fertilization.

5. Conclusion

The investigations on the structure and cytochemistry of stigma and style indicated that pistil factor is very crucial and plays a vital role in the pollen-pistil interactions and reproduction process in the angiosperms.

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