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The developmental variation of the protein profile of the pollen of *Datura inoxia*: A comparative study

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Abstract: *Datura inoxia* Mill., a less known species is generally confused with its other related species *Datura metel*, the pollen of which has been proved to be allergenic. But *D. inoxia* has been found to grow luxuriously in several places of West Bengal. There are still no reports on the allergenicity of the pollen of this plant. The present paper reports the protein profile of the pollen of *D. inoxia* at different stages of maturity. The pollen was collected both before and after anthesis and the change in protein profile and concentration studied for both immature (before anthesis) and mature (after anthesis) stages, as well as in different seasons. A slight decrease in protein concentration was observed in case of mature pollen. The SDS-PAGE protein profile showed a total of 14 protein bands designated as D1 to D14, between the molecular weight range of 22 kDa to 205 kDa. There was also a variation in the protein profile between immature and mature pollen with the number of protein bands being more in case of immature pollen.

Key words: *Datura inoxia*; immature and mature Pollen; SDS-PAGE

Introduction

Outdoor allergens, like pollen, are a cause of seasonal allergies. Inhalation allergies are elicited predominantly by pollen of various plant species. However, a classification of the large number of identified pollen allergens is still missing. Expansins, profilins, and calcium-binding proteins have been found to constitute the major pollen allergen families. Certain pollen allergens have been ubiquitous (e.g. profilins), some are present in certain plant families (e.g. pectate lyases) while some are limited to a single taxon (e.g. thaumatin-like proteins) [Radauer and Breiteneder, 2006]. The recognition of allergenic components of pollens is essential for component-resolved diagnosis, the design of patient-specific immunotherapy, and the explanation of sensitization mechanisms to various allergens (Mandal *et al.,* 2008; Valenta and Kraft, 2002)

Datura inoxia Mill. is an annual shrubby plant native to Central and South America and has been introduced in Africa, Asia, Australia and Europe. Generally, it is a low growing perennial plant, growing up to a height of about 0.5-1.5 meters. The stems and leaves are covered with short grayish hairs and the leaves are broad, elliptic, entire-edged with pinnate venation (Figure 1). The white trumpet shaped flowers are about 6-8 inches long, sometimes tinged with rose or violet and pleasantly smelled. They first grow upright and then they are inclined downwards. This plant flowers from early April till late October and can propagate easily as the fruits which are a spiny capsule split open releasing the numerous seeds. The plant tends to reseed themselves and may become invasive. Hence this plant has been found to grow as a weed in certain waste lands.

Occupational allergy constitutes a special problem, since intensive exposure to allergenic sources can result from specialized work processes. In case of *Datura*, grown in gardens as well as in greenhouses, attending the plants or selling the flowers in flower stalls brings the people concerned in close contact with and exposed to the pollen. *Datura inoxia* is one of the plant of God in India and Mexico. According to Hindu rituals, the flowers of this plant are used in the worship of Lord Shiva. Hence, they are used extensively by Hindu women to offer prayers and is a common flower sold in most road side flower stalls along with those of *Datura metel.* It also has a history as a part of puberty initiation rite in California where it is known as Toloache, in some areas being at the heart of a tribe's entire religious system.

The pollen of *Datura* sp. has been found to have a role in causing allergy in sensitive patients. Inspite of this the pollen of *Datura* sp. has till date not been considered to be a serious allergenic hazard (Parui and Mandal, 1998). One of the major reasons behind this is the entomophilous nature of the taxa. In case of pollen allergy, greater attention has been given to anemophilous pollen by aerobiologists, with the entomophilous pollen being neglected in their routine aeropalynological surveys because of their rare occurrence. Contrary to this belief, surveys have reported the presence of entomophilous pollen from the air-spora (Agashe, 1989; Agashe *et al.,* 1983, Atluri *et al.,* 1992; Singh and Babu, 1982; Singh and Devi, 1992; etc.). Moreover, pollen grains tend to be distributed in dense concentrations around their sources and therefore tend to be of local occurrence (Gregory, 1961). This is seen more in case of entomophilous pollen, which remain in

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high concentrations in air near the source plants (Durham, 1947). The pollen of *Datura* has been reported in the air by several workers (Santra *et al.,* 1991; Jain *et al.,* 1992; Chakraborty *et al.,* 2009) and the allergenic potency has been proved (Santra *et al.,* 1991; Jain *et al.,* 1992; Parui and Mandal, 1998). *Datura inoxia* pollen has still not been reported as allergenic, but with the huge resemblance with *Datura metel* which has been proved to produce allergenic pollen, it may also be responsible for allergies in sensitive patients. Being dominant and growing luxuriantly in certain parts of West Bengal where mostly females use this flower for worship, it may be difficult to evade the pollen of this plant for such sensitive patients. Unfortunately, the different species of *Datura* are found to flower round the year and the people sensitive to the pollen of this plant have very little chance to avoid this allergen.

In view of the significance of the role of pollen antigens in the diagnosis and therapy of allergenic patients, identification, isolation, purification and characterization of pollen allergens is a prerequisite for standardization. Efforts are being made globally to standardize the pollen antigens as pollen collected from different source materials, stage of purity, time intervals and geographic places or with different storage periods have shown significant variation in their allergenic components (Singh *et al.,* 1993).

The present paper reports the comparative protein profile of the pollen of *Datura inoxia* at different stages of maturity. The pollen was collected both before and after anthesis and the change in protein profile studied for both immature (before anthesis) and mature (after anthesis) stages. The variation in protein concentration was also studied during different seasons.

Materials and Methods

Pollen collection

Pollen grains of *Datura inoxia* were randomly collected in bulk from the plants growing in South Calcutta. Two types of pollen were collected - pollen from mature buds i.e. before anthesis and the other from flowers which had finished their blooming on the same day. The pollen from the anthers was sieved using different meshes (100, 200 and 300 μm). Microscopic analyses of the samples were done to check purity of 90% to 95% (Figure 2).

Protein extraction

The pollen was defatted with cold solvent ether and then dried in a vacuum desiccator. Proteins were extracted from the defatted pollen in 0.2 M Tris HCl buffer, pH 7.4 according to the method of Singh *et al.,* (1993) with slight modifications (Mondal *et al.,* 1997) by continuous stirring at 4^0C for 20h. After centrifugation at 12000xg for 5 minutes at 4^0C, the supernatant was collected. The samples were then stored at -20^0C.

Estimation of proteins

The protein concentration in the extract was estimated by the modified method of Lowry (Lowry *et al.,* 1951). A calibrated solution of bovine serum albumin was used as a standard.

Gel electrophoresis

The protein sample was heated with an equal amount of sample buffer [0.06M Tris HCl (pH 6.8), 1% SDS, 10% sucrose, 0.5% β-mercaptoethanol, 0.01% Bromophenol blue] at 100°C for 3 min. The sample was loaded in the well of a 10% T mini-gel (8x7 cm gel) and the gel was run using Laemmli buffer system (1970) [0.05 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.4] at room temperature for 1hr 20 min at 70V. The gel was calibrated using a marker mixture consisting of Myosin, Rabbit Muscle (205 kDa), Phosphorylase b (97.4 kDa), Bovine Serum Albumin (66 kDa), Ovalbumin (43 kDa), Carbonic Anhydrase (29 kDa) and Soyabean Trypsin Inhibitor (20.1 kDa). After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R250 and destained with methanol: acetic acid: water (4:1:5) mixture.

Results and Discussion

The pollen of *Datura inoxia* showed excessively high concentration of proteins in their pollen with a higher concentration in case of immature pollen than mature one (Table 1).

Table 1: Variation of protein concentration of pollen of *Datura inoxia* at different stages of maturity

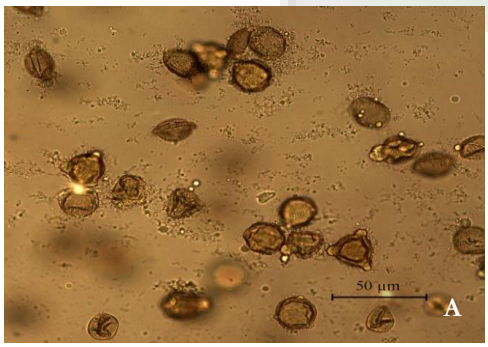
Protein concentration in immature pollen	Protein concentration in mature pollen
193.14 μg/ml	170.28 μg/ml

The SDS-PAGE protein profile of the pollen of *Datura inoxia* showed a total of 14 protein bands designated as D1 to D14 between the molecular weight ranging from 205kDa to 22 kDa. A wide variation was observed in the protein profile of mature and immature pollen. The number of the protein bands in case of immature pollen (12) was much greater than those observed in case of mature pollen (6) [Figs. 3-6]. The immature protein profile exhibited all the bands except for a 66 kDa (D9) protein which was observed only in the mature pollen. The mature pollen on the other hand showed the absence of 8 protein bands (22.4 kDa, 24.9 kDa, 56.8 kDa, 70.0 kDa, 87.0 kDa, 92.1 kDa, 183.5 kDa and 205 kDa) which were observed only in case of immature pollen (Table 2). In general, the protein content in case of immature pollen was found to be greater than that of mature pollen.

Table 2: SDS-PAGE protein profile of the pollen of *Datura inoxia*

M.W of Marker Proteins (kDa)	Proteins bands from Immature pollen		Proteins bands from Mature	
	Protein band	M.W in kDa	Protein band	M.W in kDa
205.0	D1	205.0	-	-
	D2	183.5	-	-
	D3	118.9	D3	118.9
97.4	D4	97.4	D4	97.4
	D5	92.1	-	-
	D6	87.0	-	-
	D7	82.0	D7	82.0
	D8	70.0	-	-
66.0	-	-	D9	66.0
	D10	56.8	-	-
43.0	D11	43.0	D11	43.0
	D12	32.5	D12	32.5
29.0	-	-	-	-
	D13	24.9	-	-
	D14	22.4	-	-
20.1	-	-	-	-

Figure 1 (A & B): The plant of *Datura inoxia* in full bloom.



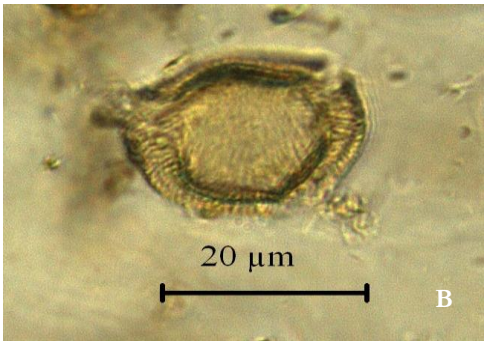


Figure 2 (A&B): The pollen of *Datura inoxia* as observed under the microscope (A) showing about 95% purity (B) single pollen.

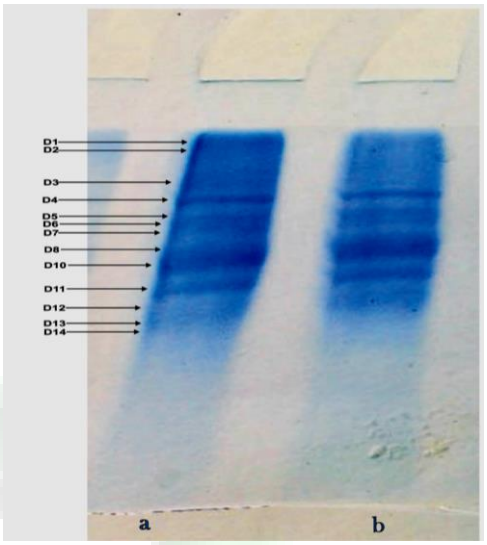


Figure 3: SDS-PAGE protein profile of the pollen of immature pollen of *Datura inoxia* (a) & (b) 5µl and 10 µl of protein sample.

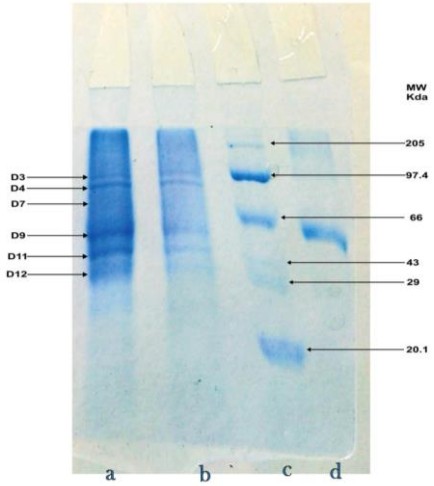


Figure 4: SDS-PAGE protein profile of the pollen of mature pollen of *Datura inoxia* (a) & (b) 40µl and 20µl of protein sample (c) molecular weight marker (d) BSA (66 kDa).

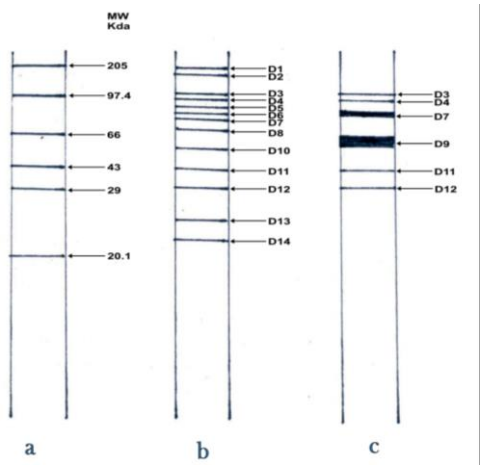


Figure 5: Diagrammatic representation of the protein profile of the pollen of *Datura inoxia* (a) Molecular weight markers (b) immature pollen (c) mature pollen.

Conclusion

Occupational allergy to *Datura inoxia* pollen may also be caused by IgE-mediated inhalation allergy. *Datura inoxia* is still not reported to produce allergenic pollen. But since it has close resemblance with *Datura metel* which is known to produce allergenic pollen, there is every possibility of similar allergenic proteins being present in the pollen of the two species. Five major proteins of *Datura inoxia* pollen have been found to be homologous to well-characterized major allergens of the closely related *Datura metel* (Bera *et al.,* 2015). These include D1 (205 kDa), D2 (183.5 kDa), D4 (97.4 kDa), D9 (66 kDa) and D11 (43 kDa). It may also contribute significantly to the aerospora. The variation in the protein contents as well as the profile with the different stages of maturity shows the need for proper standardization of the pollen extracts and designing standardized immunotherapeutic vaccines for effective allergen specific immunotherapy (AIT).

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