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RESEARCH ARTICLE

VARIATION IN THE PROTEIN PROFILE OF THE ALLERGENIC POLLEN OF *DATURA METEL* WITH DIFFERENT STAGES OF MATURITY

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ABSTRACT

Pollen of *Datura metel* has been found to have a role in causing allergy in sensitive patients. The present paper reports the comparative protein profile of the pollen of *D. metel* at different stages of development. 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. There was an increase in the protein concentration during the summer months and in general the protein content in case of immature pollen was found to be greater than that of mature pollen. The SDS-PAGE protein profile showed a total of 20 protein bands designated as d1 to d20 between the molecular weight range of 29 kDa to 205 kDa and two protein bands beyond 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.

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INTRODUCTION

Datura metel is an herbaceous, leafy annual herb growing in the wild in all the warmer parts of the world, such as India and is cultivated worldwide for its chemical and ornamental properties. It is a common herb growing along road sides, in open fields, wastelands and even grown in gardens for the erect or spreading, trumpet-shaped, 6-8 inches, pleasantly-scented flowers varying in color from white to yellow, pink, and pale purple. This plant grows up to 3 ft. It is slightly furry, with dark violet shoots and oval to broad oval leaves that are often dark violet as well. The flowers are immensely varied, and can be single or double. This plant can propagate easily as the fruits which are a spiny capsule split open releasing the numerous seeds. The plants tend to reseed themselves and may become invasive. Hence this plant has been found to grow as a weed in certain waste lands. According to Hindu rituals the flowers of this plant are used in prayers to Lord Shiva. Hence the flowers of this plant are used extensively by Hindu women to offer prayers to Lord Shiva and is, a common flower sold in most road side flower stalls.

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However the pollen of *Datura* sp. has been found to have a role in causing allergy in sensitive patients. Although having a role in allergy, this plant 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 this 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 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) and the allergenic potency has been proved (Santra *et al*., 1991; Jain *et al*., 1992; Parui and Mandal, 1998). However one has to keep in mind that although this plant can potentially cause allergies in some individuals, not everyone is affected by them. An important factor for sensitive patients is that how common and

widespread these plants are around the place where they live. With the different species of *Datura* being dominant in the different localities particularly in West Bengal, it seems impossible for the sensitive patients to avoid this allergen. An earlier study on the skin sensitivity tests with the extracts of the pollen of *Datura metel* revealed that women were almost equally affected as males and most of the affected patients were between the age group of 31-40 years, although patients were reported between the age group of 9-56 years (Parui and Mandal, 1998). Allergenic incidences were common among housewives, most of whom used the flowers of this plant on a daily basis for worship or those who cultivated this plant in their gardens either for ornamental purpose or flowers for ritual purposes. Gardeners don't let a little physical discomfort stop them from gardening. One can avoid these allergies and discomforts, if one chooses the right plants. The severity of symptoms tends to vary throughout life; many people experience periods when they have no symptoms at all. 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. Pollen allergy is caused by proteins, glycoproteins or even a single peptide which are present in the pollen wall and cytoplasm (Chanda, 1994). The soluble proteins have been generally proved to be responsible for causing nasobronchial allergy. Thus the detection of the site of origin, isolation and characterization of allergy causing proteins or glycoproteins is now a challenging task for aerobiologists working in this field (Cresti and Tiezzi, 1992). The pollen from different species not only show interspecific variation but also pollen samples of the same species collected from different source materials, stages of inflorescence, time intervals, years, different geographical places and also periods of storage, also show significant variation in their allergenic components (Singh *et al*., 1993). Thus standardization of pollen extracts is very essential for proper diagnosis and treatment of allergic diseases, as the pollen antigen of a particular species should have the same antigenic determinants every time. The present paper reports the comparative protein profile of the pollen of *D. metel* at different stages of development. 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 metel* were collected in bulk from the plants growing in South Calcutta, West Bengal. Two types of pollen were collected – one from mature buds and the other from flowers which had finished blooming on the same day as well as different days of different seasons. Pollen grains from the anthers were sieved using different meshes (100, 200 and 300 µm). All the samples were analyzed under the microscope, which revealed pollen purity varying from 90% to 95%.

Protein extraction

Proteins from pollen were extracted according to the method of Singh *et al*. (1993) with slight modification (Mondal *et al*., 1997). The pollen was defatted with cold solvent ether and then dried in a vacuum desiccator. The defatted pollen was then used for protein extraction. Proteins were extracted in 0.2 M Tris HCl buffer (pH 7.4) by continuous stirring at 4^0C for 20h. The extract was clarified by centrifugation at 12000×g 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 2hrs 30 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 R 250 and destained with methanol : acetic acid : water (4:1:5) mixture.

RESULTS AND DISCUSSION

The protein concentration showed a seasonal variation in both immature and mature pollen. There was an increase in the protein concentration during the summer months (Table 1) with the concentration increasing from 102.71 µg/ml on an average during winter to 240 µg/ml during the summer months in case of immature pollen and from 72.71 µg/ml on an average to 260 µg/ml in case of mature pollen. In general the protein content in case of immature pollen was found to be greater than that of mature pollen. The SDS-PAGE protein profile of the pollen of *Datura metel* showed a total of 20 protein bands designated as d1 to d20 between the molecular weight range of 29 kDa to 205 kDa and two protein bands beyond 205 kDa. There was also a variation in the protein profile between immature and mature pollen. The number of protein bands in case of immature pollen (20) was greater than those observed in case of mature pollen (17) (Table 2). The mature protein profile exhibited all the bands except for a 31.6 kDa (d19) protein and two protein bands beyond 205 kDa (d1 and d2) which was observed only in the immature pollen. This difference in protein profile is due to the different stage of maturation of the inflorescence from which the pollen was collected. Pollen quality has been found to vary greatly between different plant species (Roulston *et al*., 2000; Somerville and Nicol, 2006), but the causes and consequences of this variation remain unclear (Hanley *et al*., 2008). One of the longest standing hypotheses according to Lidforss (1899) is that pollen quality (usually measured as protein content) is associated with animal (entomophilous) or wind (anemophilous) pollination.

Table 1. Seasonal variations of protein concentration of pollen of *Datura metel*

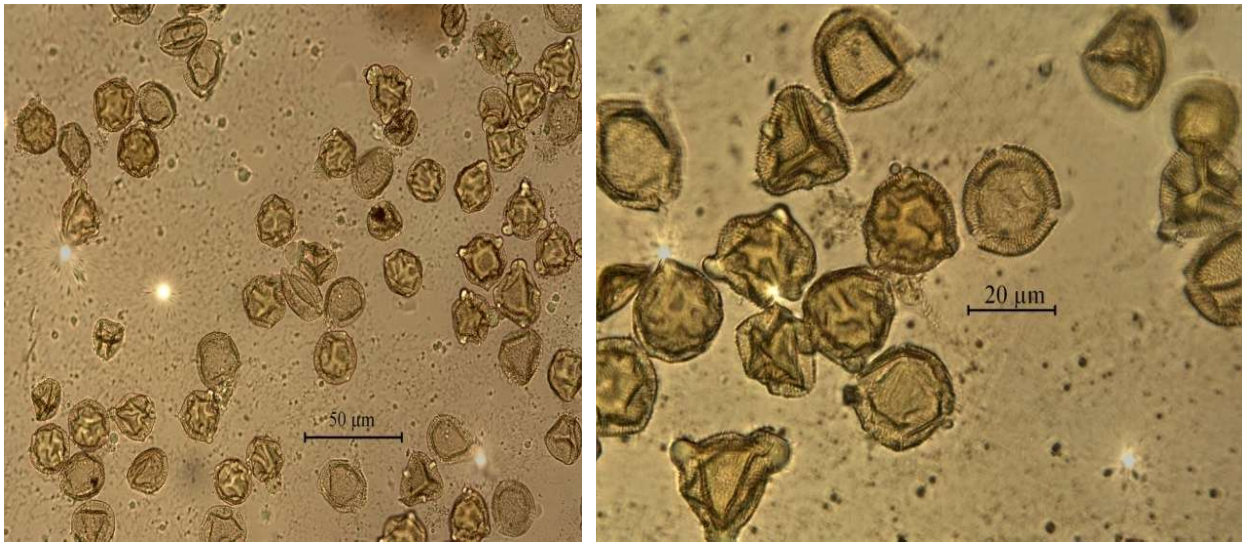
Protein concentration in immature pollen		Protein concentration in mature pollen	
September-December (Winter)	March-May (Summer)	September-December (Winter)	March-May (Summer)
102.71 µg/ml	240 µg/ml	72.71 µg/ml	260 µg/ml

Table 2. SDS-PAGE protein profile of the pollen of *Datura metel*

M.W of Marker Proteins (kDa)	Proteins bands from immature pollen		Proteins bands from mature pollen		Protein bands as observed in previous study
	Protein band	M.W in (kDa)	Protein band	M.W in (kDa)	M.W in (kDa)
	d 1	>205	-	-	-
	d 2	>205	-	-	-
205	d 3	205.0	d 3	205.0	-
	d 4	183.5	d 4	183.5	-
	d 5	172.7	d 5	172.7	-
	d 6	152.0	d 6	152.0	-
	d 7	129.7	d 7	129.7	-
	-	-	-	-	127.2
	d 8	108.2	d 8	108.2	-
97.4	d 9	97.4	d 9	97.4	97.4
	-	-	-	-	87.0
	-	-	-	-	79.1
66	d 10	66.0	d 10	66.0	66.0
	d 11	59.4	d 11	59.4	59.4
	d 12	56.2	d 12	56.2	-
	-	-	-	-	54.5
	d 13	49.8	d 13	49.8	49.8
43	d 14	43.0	d 14	43.0	-
	d 15	40.5	d 15	40.5	40.5
	d 16	37.5	d 16	37.5	37.5
	d 17	35.3	d 17	35.3	-
	d 18	33.2	d 18	33.2	-
	d 19	31.6	-	-	31.6
29	d 20	29.0	d 20	29.0	-
	-	-	-	-	18.0

A **B**

Fig. 1.


50 µm
20 µm

A B

Fig. 2.

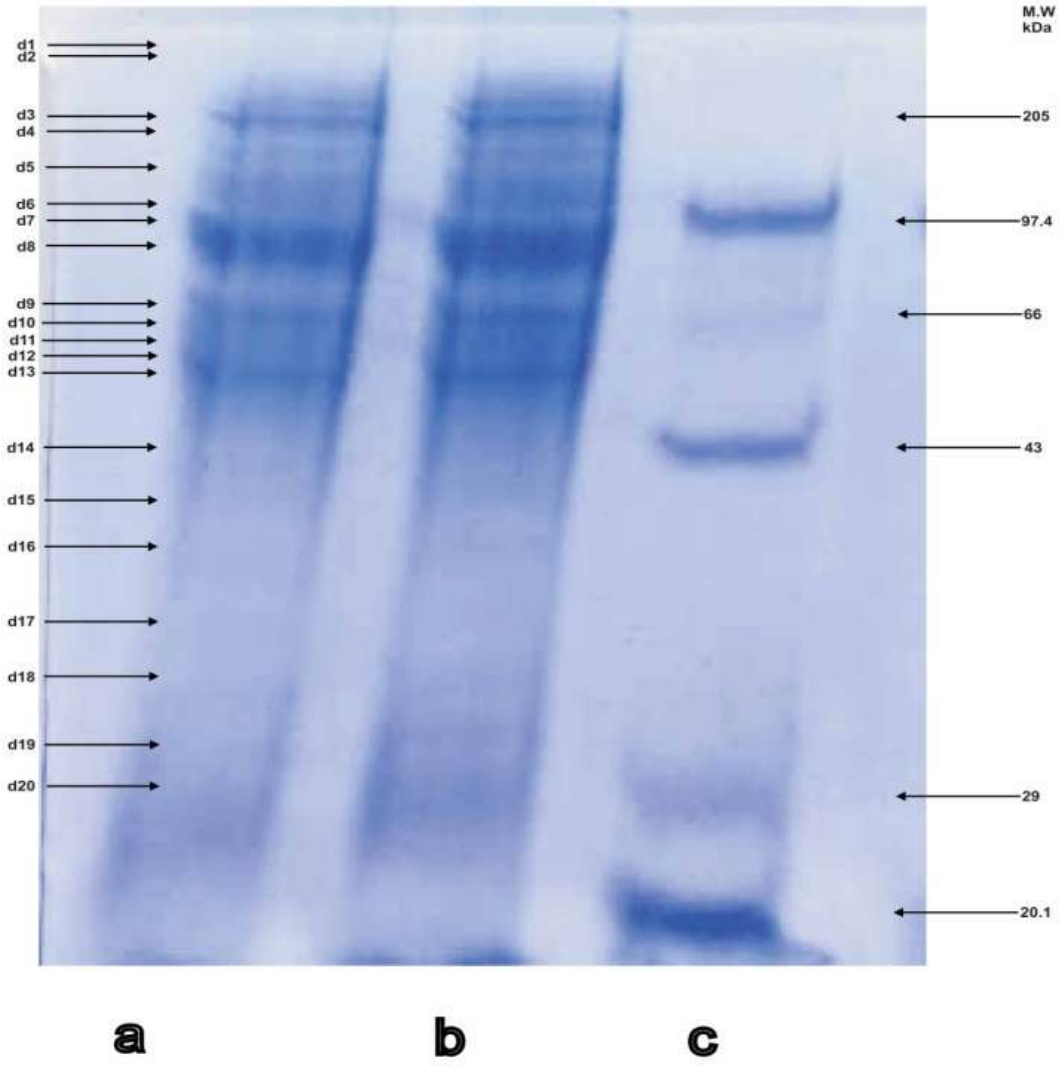

M.W
kDa
d1
d2
d3
d4
d5
d6
d7
d8
d9
d10
d11
d12
d13
d14
d15
d16
d17
d18
d19
d20
205
97.4
66
43
29
20.1
a
b
c

Fig. 3.

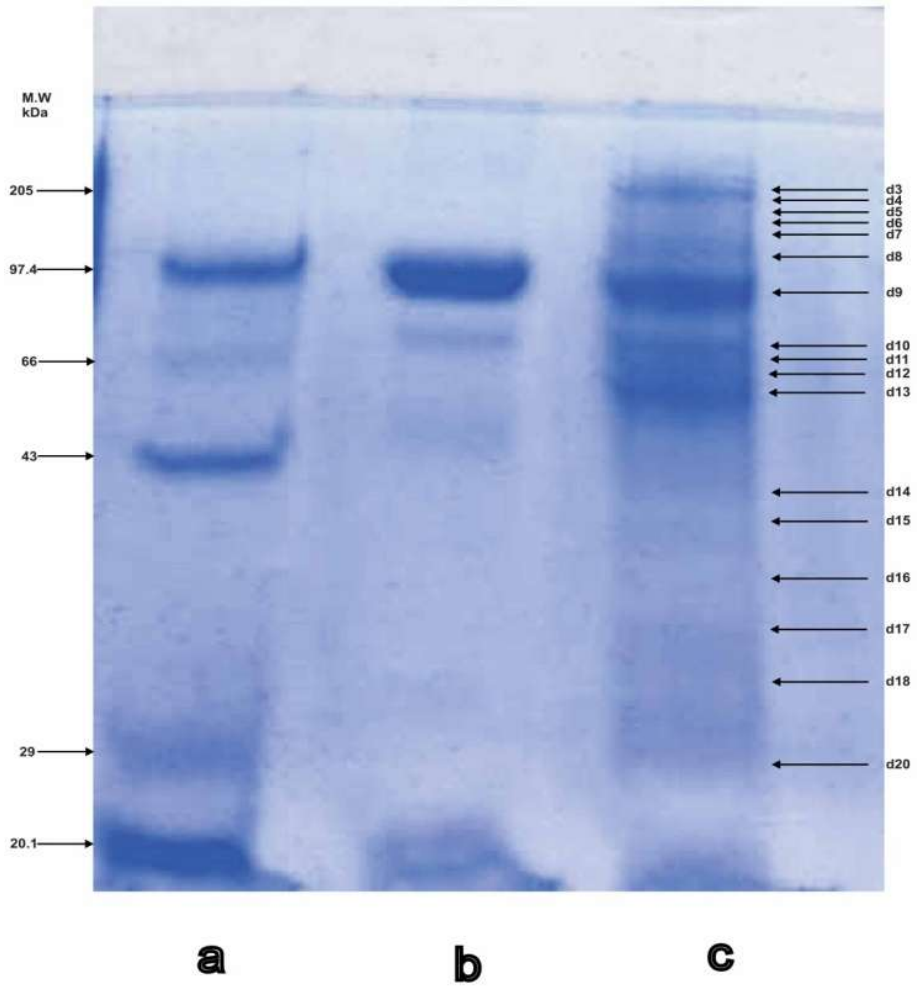

M.W
kDa
205
97.4
66
43
29
20.1
d3
d4
d5
d6
d7
d8
d9
d10
d11
d12
d13
d14
d15
d16
d17
d18
d20
a
b
c

Fig.4.

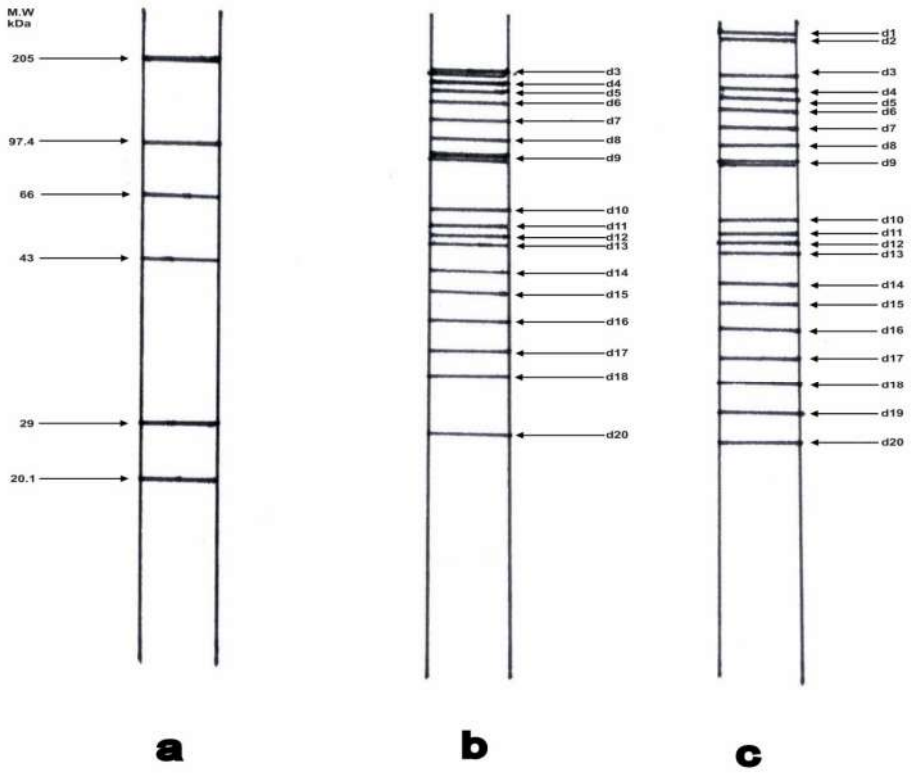

M.W
kDa
205
97.4
66
43
29
20.1
d1
d2
d3
d4
d5
d6
d7
d8
d9
d10
d11
d12
d13
d14
d15
d16
d17
d18
d19
d20
a
b
c

Fig.5.

Anemophilous plants which do not have to attract pollinators to their flowers by offering a food reward in general offer relatively low-quality pollen (Roulston *et al*., 2000). Since there are important differences in the pollination systems exhibited by entomophilous plants, and the behaviour of the pollinators that visit them, there is variation in pollen quality. This relationship between breeding system and pollen quality remains poorly explored. Some obligate entomophilous species, such as those which rely on the vibrations produced by a visiting bee to stimulate pollen release (so-called 'buzz pollination'), do seem to possess particularly protein-rich pollen (Roulston *et al*. 2000). *Datura metel* has also been found to be visited by bees. The bees (unlike nectar feeding Lepidoptera and Diptera) are entirely dependent on pollen for all of their protein requirements (Goulson, 2003; Smeets and Duchateau, 2003). However no attempts have been made to examine the relationship between pollen reward and pollinator floral preferences besides the study by Roulston *et al*. (2000). Thus, it is unclear whether there is any general trend for obligate entomophilous plants to offer higher quality pollen than plants capable of self- or wind-pollination, or whether bees preferentially visit plant species which offer higher-quality pollen.

A comparison with our earlier studies with the mature pollen of *Datura metel* collected from Santiniketan (Parui and Mandal, 1998) shows considerable variation in the protein profile. Twelve protein bands were observed between the molecular weight range of 18 kDa to 127.2 kDa. No protein bands were observed above the molecular weight range of 127.2 kDa. Seven protein bands (97.4 kDa, 66 kDa, 59.4 kDa, 49.8 kDa, 40.5 Kda, 37.5 kDa and 31.6 kDa) were common in both the studies. Five protein bands in the previous study (127.2 kDa, 87 kDa, 79.1 kDa, 54.5 kDa and 18 kDa) were not recorded in the pollen collected from Kolkata. In the present study 7 protein bands were observed above 127.2 kDa (d1- d7) which were totally absent in the pollen collected from Santiniketan. This shows that there can be a wide variation in the protein profile of pollen collected from different regions. This in turn can prove to be a serious problem for the pharmaceutical industries during the preparation of standardized immunotherapeutic vaccines.

Conclusion

Thus *D. metel* although of typical entomophilous nature and a potential allergen shows the necessity to evaluate the nature of this entomophilous pollen in proper perspective as they too contribute significantly to the air-spora as they release appreciable amount of pollen which subsequently become airborne especially close to the source and may be a matter of great concern to a sensitive individual who might show pronounced allergenic reactions (Tilak, 1989). According to Tilak (1989) concentration of entomophilous airborne pollen under favorable conditions during anthesis would vary from zero to real appreciable number. Further, the variation in the protein content 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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