ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 1998, 5, 131–134

PROTEIN PROFILE OF THE ALLERGENIC POLLEN OF *IPOMOEA FISTULOSA* L. – A COMPARATIVE STUDY

Amal K. Mondal, Sanjukta Parui, Sudhendu Mandal

Department of Botany, Visva-Barati, Santiniketan, West Bengal, India

Mondal AK, Parui S, Mandal S: Protein profile of the allergenic pollen of *Ipomoea fistulosa* L. – a comparative study. *Ann Agric Environ Med* 1998, **5**, 131–134.

Abstract: The pollen of *Ipomoea fistulosa* L. is an important aeroallergen of India. The pollen of this plant was collected from full bloomed flowers growing in different places of West Bengal, India. Protein content and profile were studied by SDS-PAGE. Skin-prick tests with pollen antigens of all the samples were also performed. Considerable variation in the protein content and profile was noted with the highest protein content in the collected sample of Calcutta showing highest number of protein bands (10) designated as IF1 to IF10 with their weights ranging between 31 kDa to 89 kDa and 3 bands (IF8, IF9 and IF10) having molecular weights less than 29 kDa. Skin-prick tests also revealed highest degree of sensitivity to the Calcutta sample giving positive response in 52% of the patients. Skin reactivity ranged between 1+ to 3+.

Address for correspondence: Amal Kumar Mondal, Department of botany, Visva-Barati, Santiniketan – 731235, West Bengal, India. E-mail: root@vbharat.ernet.in

Key words: Pollen, *Ipomoea fistulosa*, different geographical regions, SDS-PAGE, skin-prick tests.

INTRODUCTION

It is well known that there has been a gradual increase in the number of people suffering from allergy and other respiratory diseases. About 15–20% of the world's population are suffering from allergic disorders i.e. allergic rhinitis, bronchial asthma, atopic dermatitis and urticaria [12]. According to a survey by Kjellman [5], one child out of every three, has a history of allergy or atopic disease before reaching the age of seven to ten years. Among the various aeroallergens, the role of pollen in causing respiratory disorders in sensitive patients has been greatly realized. Pollen allergy is caused by proteins, glycoproteins or even a single peptide which are present in the pollen wall and cytoplasm [1]. 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 very challenging task for aerobiologists working in this field [2]. Pollen samples collected from different source materials, stages of inflorescence, time intervals, years, different geographical places and also periods of storage, show significant variation in their allergenic components [11]. Thus, standardization of pollen extracts is very essential for proper diagnosis and treatment of allergic diseases, as the pollen antigen of a particular species could have the same antigenic determinants every time.

In the present investigation, pollen of *Ipomoea fistulosa* L. was collected from four regions of West Bengal, i.e. Santiniketan, Burdwan, Calcutta and Siliguri, located approxi-mately 59 km, 146 km and 447 km from Santini-ketan. The protein content and profile of all the samples were studied to discover the variation in the allergenic components of pollen samples collected from different geographical regions and different source materials. The pollen antigens were also prepared and skin test performed with all the samples to detect any variation in their allergenic potential.

Received: 5 March 1998
Accepted: 25 September 1998

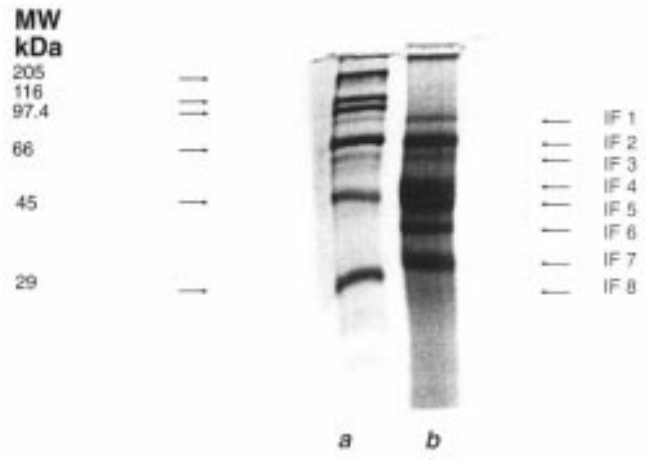

Figure 1. SDS-PAGE of pollen extract of *I. fistulosa* collected from Santiniketan: (a) represents marker proteins, (b) contains 84 µg of crude homogenate.

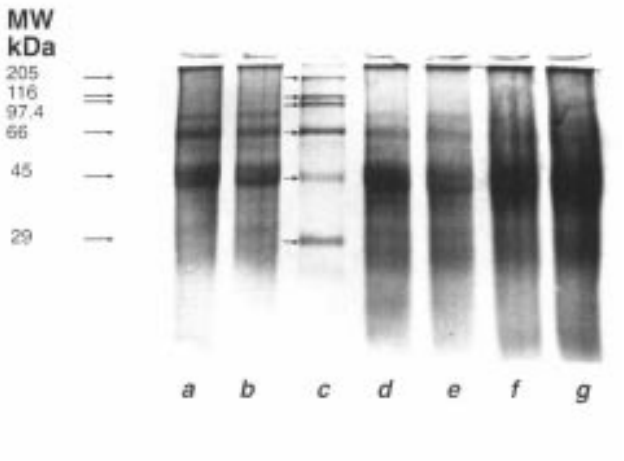

Figure 2. SDS-PAGE of pollen extracts of *I. fistulosa* collected from: (a, b) Burdwan, (d, e) Calcutta, (f, g) Siliguri and (c) represents marker proteins.

MATERIALS AND METHODS

Pollen grains of *Ipomoea fistulosa* were collected in bulk from the plants growing in the four places of West Bengal i.e. Burdwan, Calcutta, Santiniketan and Siliguri, from full bloomed flowers. Pollen from the anthers were sieved using different meshes (100, 200 and 300 µm). Microscopic analysis of all the samples were carried out which revealed pollen purity varying from 85% to 90%.

Table 1. Protein content of pollen extracts of *I. fistulosa* form different places.

Place of collection	Protein (mg ml^{-1})
Burdwan	7.6
Calcutta	9.2
Santiniketan	8.4
Siliguri	8.6

Table 2. Molecular weights of various protein bands of the antigenic extract of *I. fistulosa* from different places.

Fraction	Burdwan	Calcutta	Santiniketan	Siliguri
		(M.W. in kDa)		
IF1	89	89	89	89
IF2	68	68	68	68
IF3	57	57*	57	absent
IF4	47	47	47	47
IF5	42	42	42	42
IF6	absent	33*	33	33*
IF7	31	31	31	31
IF8	<29**	<29**	<29**	<29**
IF9	absent	<29	absent	absent
IF10	absent	<29	absent	<29

* poorly developed, ** very well developed

Protein from the pollen of all four samples were extracted in 0.2 M Tris HCl buffer, pH 7.4 by continuous stirring at 4ºC for 20 h according to the method of Singh *et al.* [11]. The extract was clarified by certrifugation at 15,000 g for 25 min at 4ºC. The supernatant was used for quantifying the protein as well as for studying the protein profiles. The protein concentration in all the samples was estimated by the method of Lowry *et al.* [7]. A calibrated solution of bovine serum albumin was used as standard. SDS-Polyacrylamide Gel Electrophoresis was performed using a 10% T mini-gel (8 × 7 cm gel) following the method of Laemmli [6] to determine the protein profiles. The gel was calibrated with a marker mixture (MW range between 29 kDa to 205 kDa) obtained form Sigma Co., USA. 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. Pollen antigens from all the samples were extracted according to the method of Sheldon *et al.* [10] and the allergenic potential of each molecular sample measured by skin prick tests performed at the Calcutta Medical Research Institute, Calcutta.

RESULTS

The protein content and profile of the pollen of *Ipomoea fistulosa* collected from different regions showed considerable variation. The concentration of protein varied between 8.4 mg ml^{-1} to 9.2 mg ml^{-1} with the highest concentration observed in the sample collected from Burdwan (Tab. 1). The SDS-PAGE protein profiles revealed 10 bands which were designated as IF1 to IF10. The pollen samples of Burdwan, Calcutta and Siliguri revealed 7, 10 and 8 bands respectively (Tab. 2; Fig. 1, 2, 3). The sample collected from Burdwan showed the absence of IF6 (33kDa) while IF8 (< 29 kDa), which was present as a faint band in the Santiniketan sample, was very well developed. The Calcutta sample revealed the largest number of bands, with two extra bands below IF8 which were designated as IF9 and IF10 (molecular weights < 29 kDa).

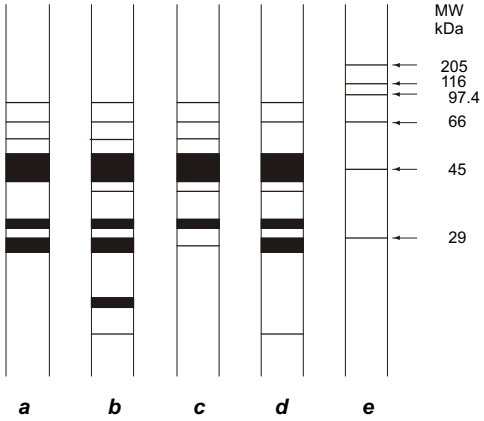

Figure 3. Diagramatic representation of the pollen extracts of *I. fistulosa* collected from different places of West Bengal: (a) Burdwan, (b) Calcuta, (c) Santiniketan, and (d) Siliguri; (e) marker proteins.

IF3 (57 kDa) and IF6 (33 kDa) were poorly developed in this sample. The antigenic extracts of the pollen collected from Siliguri revealed 8 bands with the absence of IF3 (57 kDa) and IF9 (< 29 kDa) while IF6 (33 kDa) was weakly developed and IF8 (< 29 kDa) present as a very thick band.

Variation in skin sensitivity was also observed in the different samples (Table 3). Skin prick tests with the extracts of pollens sampled in different locations were performed in 35 patients already suffering from respiratory allergic disorders, with ages ranging between 9-71 years. Among the heterogenous population showing positive reaction to total extract, 23 (65.7%) were atopic, of whom 3 patients had suffered from asthma for 17-28 years and 5 from allergic rhinitis for 9-14 years. The highest degree of skin reactivity was observed in the case of the Calcutta sample with 18 patients (51.4%) showing positive reactions, of whom 6 (17.1%) showed 1+ reaction, 7 (20.0%) showed 2+ reaction, while 3+ reaction was obtained in 5 patients (14.3%). The lowest degree of skin reactivity was observed in case of the Burdwan sample with 13 patients (37.1%) reacting positively and only 2 (5.7%) showing 3+ reaction. This was followed by the sample collected in Santiniketan, showing positive response in 15 patients tested (42.8%), while Siliguri sample producing 1+ to 3+ reaction in 17 patients (48.6%).

Table 3. Results of skin tests with the antigen of *I. fistulosa* from different places.

Place of collection	Total number of tests	Reaction of patients (%)			
		negative	1+	2+	3+
Burdwan	35	63	21	10	6
Calcutta	35	48	16	21	15
Santiniketan	35	57	14	20	9
Siliguri	35	50	15	21	14

Diameter of erythema (mm): 0: no reaction, 1–5: 1+, 6-10: 2+, 11-15: 3+.

DISCUSSION

The pollen of *Ipomoea fistulosa* proves to be one of the important aeroallergens of West Bengal capable of inducing respiratory allergic diseases in India. The protein profile of the pollen of *Ipomoea fistulosa* collected form Santiniketan has been reported earlier [8] in which eight bands were obtained (IF1 to IF8), the molecular weights of which ranged between 31kDa and 89 kDa, with the band IF8 having a molecular weight range below 29 kDa. The differences in the protein profiles, as well as the protein content in the samples collected from the various regions of West Bengal, could be due to variable climatic conditions prevailing in these areas and soil conditions, as has been earlier suggested by Singh *et al.* [11]. The highest rate of skin sensitivity in the sample collected from Calcutta may be due to the higher protein content as well as the additional protein bands (IF9 and IF10), resulting in sensitivity to some patients due to these protein fractions. The lower degree in sensitivity in the samples from other places may be due to the absence or low intensity of certain bands. Another factor for increasing the allergenicity of pollen is air pollution. This is effected by changing the kind and proportions of exinic mineral elements [3, 13], or by affecting pollen morphology or protein profile [4, 9]. Thus, the atmosphere of Calcutta, being the most polluted due to industrialization and vehicular traffic, may be responsible for the increased rate of allergenicity of the pollen grains.

CONCLUSION

The results of the present investigation therefore confirms that:

- Considerable difference in profile occurs in the pollen samples collected form different geographical regions, thus proving that pollen authenticity, source, method of storage, handling and extraction play a very important role in the standardization of pollen extracts used in immunotherapy.
- The variation in skin reactivity to extracts of pollen samples from different regions proves the heterogeneity in the specific IgE binding patterns of different individuals.
- The higher degree of sensitivity in the Calcutta sample may be presumed due to the high degree of inorganic pollutants in the atmosphere of that region, thus making it very difficult to determine the actual causative agent for the allergenic responses in human system when the pollution level in the atmosphere is significantly high.

Acknowledgement

The authors are indebted to the Council of Scientific and Industrial Research, New Delhi, for their financial support, and Calcutta Medical Research Institute, Calcutta, for carrying out the skin tests.

REFERENCES

1. Chanda S: Pollen Grains as Aeroallergens: Morphological, Biological and Chemical Approach. **In:** Agashe SN (Ed): *Recent Trends in Aerobiology, Allergy And Immunology*, 85-92. Oxford & IBH Publ Co Pvt ltd, New Delhi 1994.
2. Cresti M, Tiezzi A (Ed): *Sexual Plant Reproduction*, 203-217. Springer Verlag Berlin 1992.
3. D'Amato G: *Allergenic Pollen and Pollinosis in Europe.* Blackwell Scientific Publ Ltd 1991.
4. Dass A , Singh A, Singh AB: Impact of exposure of SO_2 on pollen morphology and its protein profile. **In:** *29th Ann Conv Coll Aller & Appl Immunol*, Calcuta 1995, Abst. 13.
5. Kjellman NIM: Prediction and prevention of allergy in infants and children. *Allergy Clin Immunol News* 1993, **5**, 131-134.
6. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, **227**, 680-685.
7. Lowry OH, Roseborough NJ, Farr AL, Randall RJ: Protein measurement with Folin phenol reagent. *J Biol Chem* 1951, **193**, 265-273.
8. Mondal AK, Parui S, Biswas SR, Mandal S: Identification o the allergenic proteins of *Ipomoea fistulosa* L. pollen – Partial characterization & sensitivity test. *Grana* 1997, **36**, (in press).
9. Santra SC, Gupta S, Chanda S: Air pollutant and aeroallergens interaction. *Grana* 1991, **30**, 63-66.
10. Sheldon JM, Lovsell RG, Mathews KP (Ed): *A Manual of Clinical Allergy*. WB Saunders Co, Philadelphia 1967.
11. Singh AB, Malik P, Parkash D, Gangal SV: Identification of specific IgE binding proteins in Castor bean (*Ricinus communis*) pollen obtained from different source materials. *Grana* 1993, **31**, 376-380.
12. Singh AB, Singh A: Pollen Allergy: A Global Scenario. **In:** Agashe SN (Ed): *Recent Trends In Aerobiology, Allergy And Immunology*, 143-170. Oxford & IBH Publ Co Pvt Ltd, New Delhi, Bombay, Calcutta 1994.
13. Solomon WR: Aerobiology of pollinosis. *J Allergy Clin Immunol* 1986, **74**, 449-461.