

Allergenic relationship among four common and dominant airborne palm pollen grains from Eastern India

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Summary

Background Palm pollen grains are predominant aeroallergens in the tropics including India. Evidence of allergenic crossreactivity had been reported from various parts of the world on different families, e.g. Poaceae, Asteraceae, etc. No such information is available about the palm pollen of tropical countries.

Objectives The present study was undertaken to find out the allergenic relationship, if any, in four common and important palm pollen in India.

Methods A 2-year aerobiological survey was carried out at Madhyamgram situated at the suburban fringe of Calcutta Metropolis using Burkard volumetric sampler to know the seasonal variation of *Areca catechu*, *Borassus flabellifer*, *Cocos nucifera* and *Phoenix sylvestris* among others. Skin-prick tests (SPT) were performed with the relevant pollen extracts on the respiratory allergic patients. Sera from the subjects were tested directly by ELISA for estimating the allergen specific IgE. ELISA inhibitions and dot blotting were performed with pooled patients sera and four palm pollen extracts to detect the cross-reactivity.

Results Among 70 patients, *Areca catechu* exhibited the maximum percentage (48.5%) of positive responses followed by *Cocos nucifera* (45.7%), *Phoenix sylvestris* (42.85%) and *Borassus flabellifer* (38.5%). On an average, 30–50% of the patients showed positive skin reactions and enhanced specific IgE level to more than one palm pollen extract. Further evidence of allergenic crossreactivity among the four palm pollen grains were provided by dot blotting and ELISA inhibition studies. In ELISA inhibition, a distinct inhibition was obtained with comparable amounts of the pollen extracts.

Conclusion The suburban aerobiological survey of Calcutta and SPT results confirmed that the relevant pollen types are significant contributors of aeroallergen load of the study area. ELISA inhibition studies with the pooled patients sera using antihuman IgE probe revealed the presence of shared allergenic components among the four palm pollen grains.

Keywords: pollen allergy, palm pollen, crossreactivity

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Introduction

Pollen allergens have been reported to be important triggers of IgE mediated allergic disorders, e.g. rhinitis or asthma. The study of these allergenic molecules is an area of interest for allergy diagnosis and therapy. Quite a number of plants

producing allergenically potent pollen have been reported from both tropical and subtropical parts of India [1–3]. It has been reported that about 10% of the people living in the city of Calcutta suffer from different types of respiratory disorders. An airborne pollen survey was made for two consecutive years (July 1994 to June 1996) using Burkard volumetric air sampler in the Madhyamgram Field Station of Bose Institute, a suburban area, 19 km north from Calcutta, on the basis of which a pollen calendar was prepared. In the survey, out of 46 recorded pollen taxa,

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four palm pollen types were found to be frequent in air and were placed in the list of first eight according to allergenic potential checked by SPT [4]. They were *Areca catechu* L. (areca palm), *Borassus flabellifer* L. (pal Myra palm), *Cocos nucifera* L. (coconut palm) and *Phoenix sylvestris* Roxb. (date sugar palm), which play an important role in Indian economy and are cultivated or wildy grown in different parts of the country. The relevant pollen types were found to be allergenically potent by skin reaction test [5] in earlier reports. Out of these four types, only coconut pollen allergen was studied in detail [6–8]. Although allergenic cross-reactivity among the pollen grains of taxonomically related [9–11] and unrelated [12] species were reported, clinical and biochemical investigation on palm pollen allergenicity did not receive attention. The relevant pollen grains originate from four taxa of the family Arecaceae, they are good pollen producers, wind pollinated and allergenically active, enough criteria to undertake this investigation.

Materials and methods

Pollen count

An aerobiological survey was carried out using a Burkard volumetric pollen sampler highlighting occurrence and frequency of relevant palm pollen grains. The sampling period extended from July to June, 1994–96. The volumetric sampler was placed on an agricultural field at Madhyamgram, 19 km north from Calcutta. The Burkard tape was regularly scanned microscopically as per guideline of The British Aerobiological Federation [13] to study the seasonal variation of relevant airborne pollen grains.

Preparation of pollen extract

Fresh pollen samples were collected from the mature buds of the relevant plants. The pollen samples containing >90% pure pollen were defatted with diethyl ether and the extracts were prepared in phosphate buffered saline (PBS, 0.1 m Na-phosphate containing 0.15 m NaCl, pH 7.2) by stirring at 4°C for 24 h. The extracts were centrifuged at 12 500 *g* for 40 min and the supernatant was brought to 90% saturation with ammonium-sulphate. The precipitate was dissolved in PBS and dialysed (mol. wt. cut limit 10 000). The dialysed extracts were filtered through 0.22 µm millipore filter (Millipore, Bedford, MA, USA). The filtrate was stored at –20°C in sterile vials.

Protein determination

The protein content of each extract was determined by the method of Lowry *et al.* [14] using BSA as standard.

Skin tests

Skin tests were performed by the skin-prick technique with the relevant pollen extracts on 70 adult respiratory allergic patients attending Allergy Unit of the Institute of Child Health, Calcutta. The reaction was graded 1+ to 3+ after 20 min as described by Stytiš *et al.* [15] using histamine diphosphate (1 mg/mL) and PBS as positive and negative controls, respectively.

Collection of sera

Sera were collected from the patients showing positive

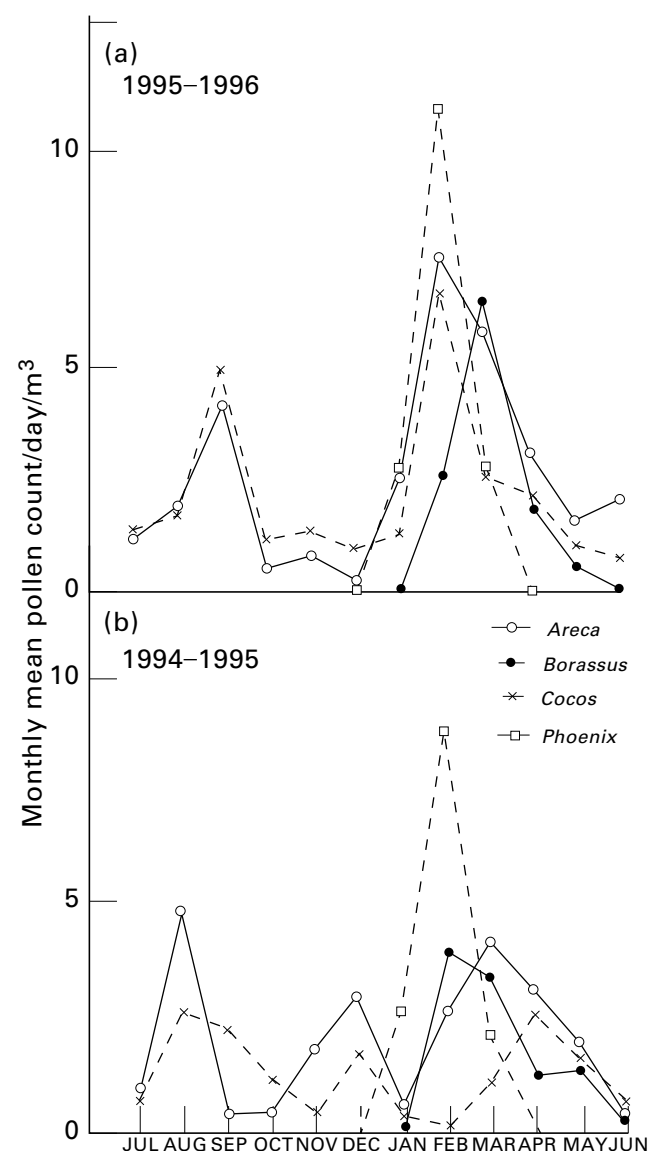


Fig. 1. Variation of average monthly concentration of four common airborne palm pollen grains.

(>2+) skin reaction to any of the four palm pollen tested, before they were administered for immunotherapy. Normal human sera (NHS) obtained from nonallergic subjects showing negative skin reaction were used as control. Consent of all patients was taken prior to skin test and serum collection.

Enzyme Linked Immunosorbent Assay (ELISA)

ELISAs were performed to measure the specific IgE levels in individual patient sera for each palm pollen extract using antihuman IgE peroxidase (1:1000) conjugate (Sigma Chemical Co., USA) and o-phenylene diamine substrate [16]. The absorbance was measured at 492 nm with an ELISA reader. The individual patient sera having P/N (the ratio of OD of patient serum with respect to normal human serum) value > 3.5, considered as significant, were pooled for ELISA inhibition studies.

IgE specific dot blotting

Dot blotting was performed on the pieces (2 x 2 cm²) of nitrocellulose membrane to observe the IgE specific cross-reactions [17]. The peroxidase positive spots were detected using diaminobenzidine [18] as substrate.

ELISA inhibition using anti-human IgE enzyme complex

ELISA inhibition [19] was performed by incubating 100 µL of pooled patients sera with 100 µL of inhibitor (palm pollen extracts) in different concentrations for overnight at 4 °C. The inhibited serum was then added to the wells previously coated with antigen. The antibodies bound to the plate were detected after overnight incubation by anti-IgE peroxidase

enzyme labelled conjugate. Per cent inhibition was calculated as:

% Inhibition =

$$\frac{\text{OD of test sample} - \text{OD of inhibited sample}}{\text{OD of the test sample}} \times 100$$

The point of 0% inhibition was obtained by incubating the serum pool with PBS. A dose-response curve was obtained by plotting the percentage inhibition against allergen concentration. From this curve the quantitative value of each allergen for 50% inhibition was determined.

Results

Aerobiological survey

Figure 1 represents the monthly variation patterns of the four palm pollen grains concentration during the period of July 1994 to June 1996. The *Areca* and *Cocos* pollen were found to be present throughout the year, i.e. they were perennial. Presence of *Borassus* pollen was mainly restricted to the months of February to May and maximum pollen counts were recorded in February 1995 and March 1996. *Phoenix* pollen appeared mainly in the months of January, February and March, with a predominance in February.

Skin-prick tests and ELISA

Among the four palm pollen grains, *Areca catechu* exhibited the maximum percentage (48.5%) of positive response in a total test population of 70 patients sensitive to any one or more of the relevant palm pollen followed by *Cocos*

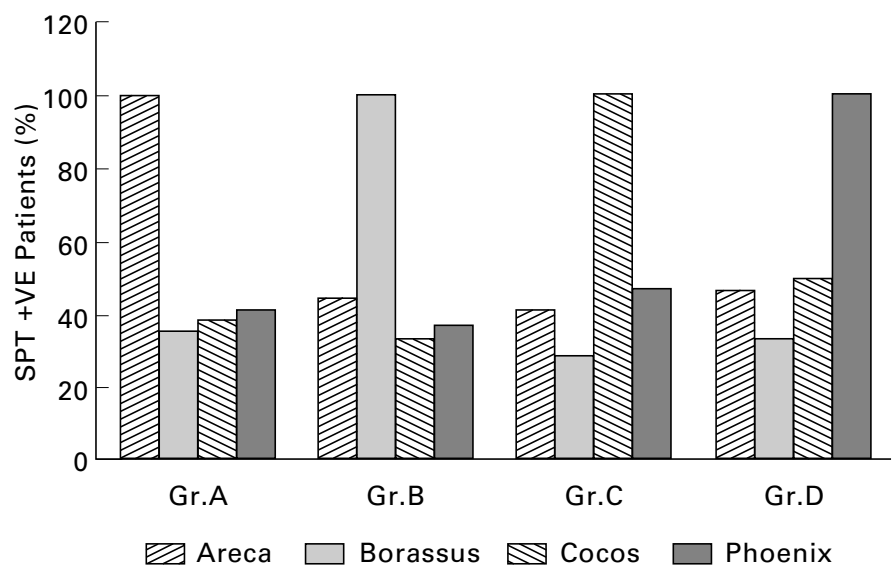


Fig. 2. Comparative results of SPT with four palm pollen extracts in 70 allergic patients. Per cent positivity (Y axis) to other three pollen extracts in *Areca* positive patients (Gr.A, *n* = 34); *Borassus* positive patients (Gr.B, *n* = 27); *Cocos* positive patients (Gr.C, *n* = 32) and *Phoenix* positive patients (Gr.D, *n* = 30).

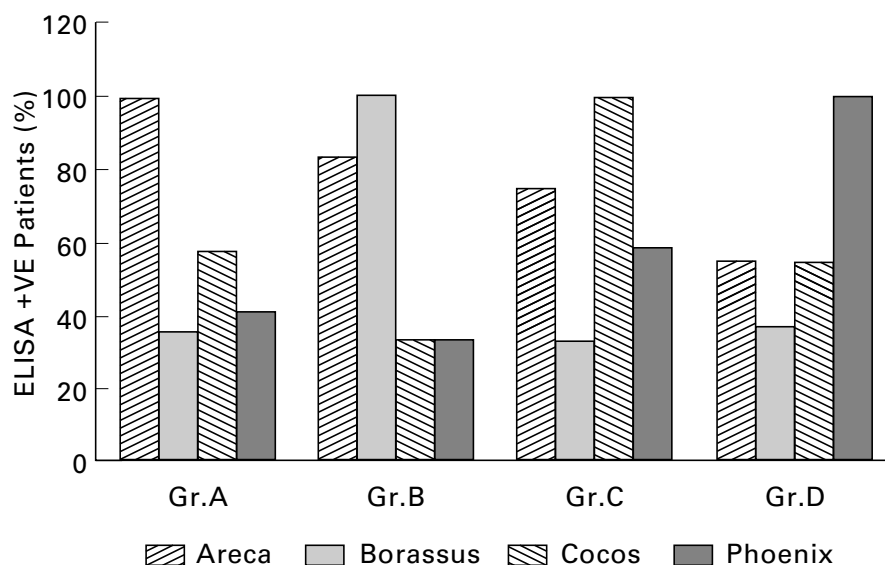


Fig. 3. Comparative account of ELISA results with 35 patient sera positive to palm pollen extracts. Per cent positivity (Y axis) to other three palm pollen extracts in *Areca* positive patients (Gr.A, $n = 12$); *Borassus* positive patients (Gr.B, $n = 6$); *Cocos* positive patients (Gr.C, $n = 12$) and *Phoenix* positive patients (Gr.D, $n = 11$).

nucifera (45.7%), *Phoenix sylvestris* (42.85%) and *Borassus flabellifer* (38.5%). Figure 2 shows the comparative analysis of the skin-prick test results with four palm pollen extracts in 70 allergic patients. About 40% of the patients sensitive to *Areca* pollen showed positive response to allergenic extracts of *Phoenix* and *Cocos*. Among *Borassus* positive patients 33% to 44% elicited positive skin reaction to the other three pollen extracts. In *Cocos* sensitive patients, >40% subjects showed positive reaction to *Phoenix*, followed by *Areca* and *Borassus*. Similarly in *Phoenix* positive patients, the percentage of marked positive response in skin reaction was more in *Cocos* than *Areca* and *Borassus*.

Out of 70 skin tested patients with four palm pollen

extracts, blood was drawn from 35 patients with their previous consent for further study. The serum of each patient was tested individually against all the four palm pollen extracts to detect the presence of specific IgE. The result of ELISA was expressed in terms of P/N-value and the percentage of the patients showing positive responses in ELISA to one extract was taken as 100% and compared with the values of the other three extracts (Fig. 3).

IgE specific dot blotting

The dot immunoblotting assay was performed to test the crossreactivity of four palm pollen extracts to the serum pool prepared from the patients individually sensitive to any

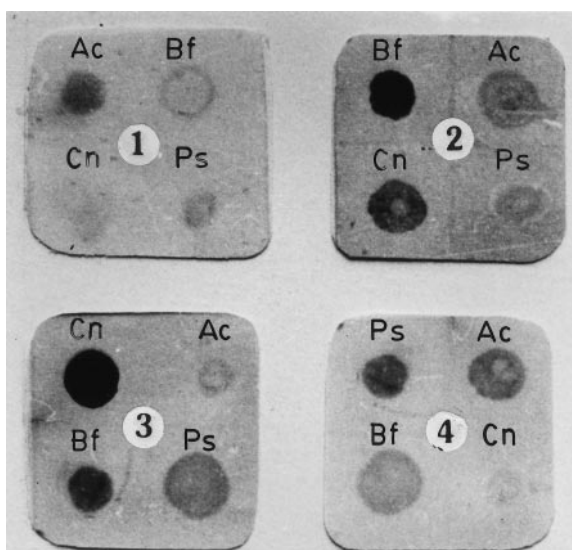


Fig. 4. Detection of allergenic relationship among four common palm pollen extracts by IgE specific dot immunoblotting with allergic patient sera. The sera used in four nitrocellulose pieces were collected from: 1. *Areca*, 2. *Borassus*, 3. *Cocos* and 4. *Phoenix* sensitive patients. The spots were marked as Ac = *Areca*, Bf = *Borassus*, Cn = *Cocos* and Ps = *Phoenix* pollen extracts.

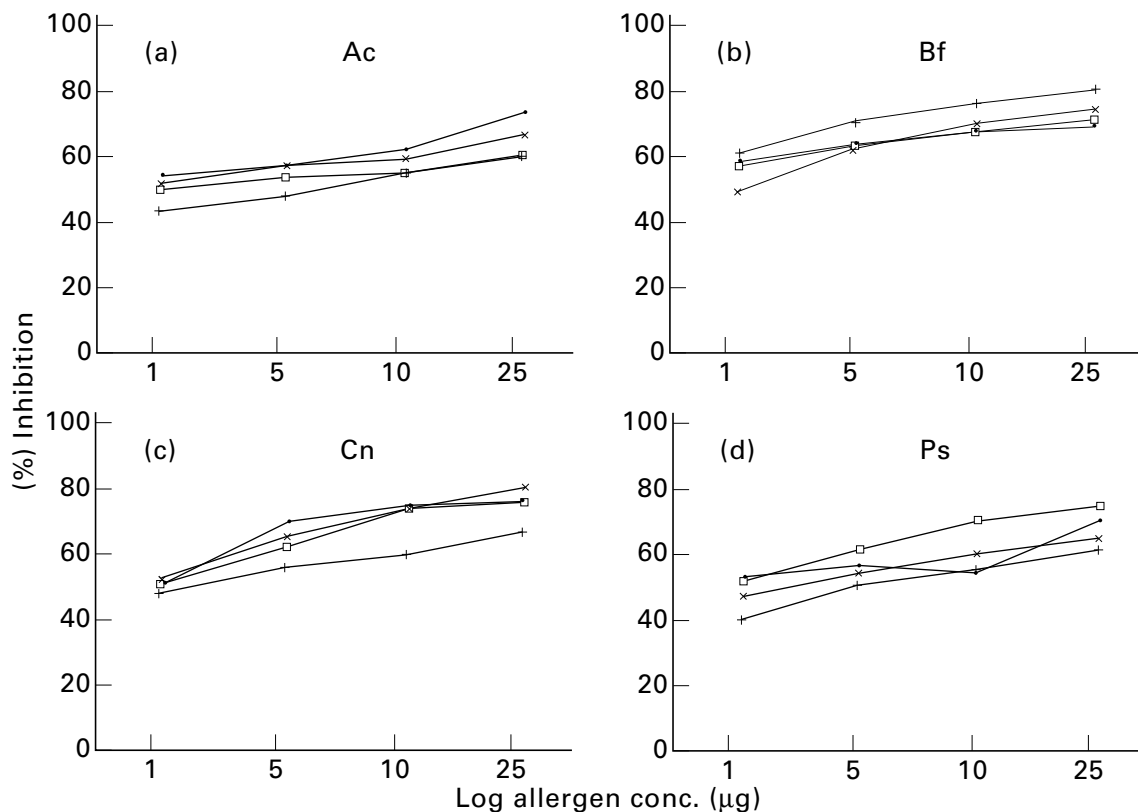


Fig. 5. ELISA inhibition demonstrating allergenic crossreactivity among four palm pollen extracts. Solid phase used are: Ac = *Areca catechu*, Bf = *Borassus flabellifer*, Cn = *Cocos nucifera* and Ps = *Phoenix sylvestris*. The palm pollen extracts used as inhibitors for IgE binding in patient sera are: *Areca* —+, *Borassus* —●—, *Cocos* —x— and *Phoenix* —□—.

one of the palm pollen extracts (Fig. 4). The serum pool against each relevant pollen recognized allergenic determinants in all the four pollen extracts. A strong binding was observed with the homologous allergens. The result was heterogeneous with the heterologous allergen extracts.

ELISA inhibition using anti-IgE probe

Further evidence of allergenic crossreactivity among the four palm pollen grains was observed by competitive ELISA

Table 1. Allergenic crossreactivity among the four palm pollen extracts as measured by ELISA inhibition with palm pollen sensitive patient sera

Solid phase	Allergen used for inhibition			
	<i>Areca</i>	<i>Borassus</i>	<i>Cocos</i>	<i>Phoenix</i>
Allergen <i>Areca</i>	<1	4.5	<1	1.0
<i>Borassus</i>	<1	<1	1.5	<1
<i>Cocos</i>	1.2	2.5	<1	1.0
<i>Phoenix</i>	<1	4.8	2.4	<1

* Amount (μg) required for 50% inhibition.

inhibition using a serum pool of each allergen extract and anti-IgE peroxidase conjugate. A clear-cut inhibition of IgE binding specific to the four palm pollen allergen extracts was obtained with comparable amounts of the extracts. Interestingly, it was noted that not only the homologous extracts but also the heterologous extracts produced significant dose dependent inhibition in the range (1–25 μg) tested (Fig. 5). The amount of each allergen required for 50% inhibition was calculated and presented in the Table 1.

Discussion

The study of airborne pollen grains is important because of its application in diagnosis and treatment of patients suffering from respiratory allergic diseases. Among the different types of allergenically active pollen grains, palm pollen grains were also found to be allergenic through aerobiological and clinical survey conducted in urban areas of Calcutta [5,6]. Among the four studied palm pollen types, *Borassus* and *Phoenix* were found to be seasonal and the presence of airborne pollen corroborated with their flowering periods. The other two pollen types, i.e. *Areca* and *Cocos*, were perennial flowering throughout the year. In the

respective peak periods of these pollen types, *Areca*, *Borassus*, *Cocos* and *Phoenix* contribute 7.93%, 8.42%, 8.99% and 15.40%, respectively, in terms of the total pollen load in the air. Here the pollen concentration varied according to different meteorological parameters, which was not highlighted here. These results have turned out to be very much similar to the observation made previously in Salt Lake City of Calcutta during 1988–90 [20].

In a random survey of 551 patients undergoing skin-prick tests for allergy diagnosis in the Institute of Child Health, Calcutta, 241 (55.89%) patients were found to be positive to one or more palm pollen extracts tested. Among them, 70 patients were selected for repeat tests with the four palm pollen extracts. Although the *Areca* pollen responded to maximum percentage (48.5%) and *Borassus* the least (38.5%), the differences were not remarkable. The majority of the individuals suffering from palm pollen allergy reacted to more than one palm pollen extracts (Fig. 2). ELISA was performed to evaluate again the allergenic crossreactivity among them. Analysis of the positive ELISA values for one palm pollen extract in comparison to the other three showed elevated IgE levels to more than one allergen in many patients. The SPT and ELISA results apparently showed that the patients were getting sensitized primarily against the palm pollen which flowered round the year and the reaction against the other two seasonal palm species was simply due to crossreactivities. However, there were some patients who responded solely to any one of the seasonal types.

The result of dot blotting analysis, which is a simple and reliable technique, indicated the presence of crossreacting allergens in the relevant palm pollen extracts.

ELISA inhibition was performed in order to quantify the extent of crossreactivity using pooled sera and serial dilutions of the four extracts (Table 1 and Fig. 5) although the inhibitory capacity of the extracts was found to be different depending on the serum pool and the solid phase allergens used, the nature of curves were very much similar. This may be due to the presence of some common allergenic proteins in these palm pollen extracts.

These findings with the palm pollen allergens further enrich the general thinking of the presence of common allergenic components in the pollen grains of taxonomically closely related plant species [10,21,22].

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