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ANTIGENIC RELATIONSHIP BETWEEN FOUR AIRBORNE PALM POLLEN GRAINS FROM CALCUTTA, INDIA

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Abstract: The pollen grains of *Areca catechu, Borassus flabellifer, Cocos nucifera* and *Phoenix sylvestris,* all belonging to the family *Aracaceae* (*Palmae*), are airborne and found to be potent in causing human respiratory allergy. The present study was undertaken to discover the antigenic relationship, if any, in the four relevant palm pollen grains. The study was conducted by using *Borassus* and *Phoenix* antisera raised in rabbit. These antisera were used in rabbit IgG specific ELISA-inhibition and rocket immunoelectro-phoresis (RIE) assays for all four palm pollen extracts. In ELISA-inhibition, a distinct inhibition was obtained with comparable amount of soluble pollen protein. The RIE precipitin bands also revealed the presence of common antigenic components in the palm pollen. After isolation and purification, such common antigens may be useful in allergen immunotherapy in asthmatics.

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INTRODUCTION

Pollen grains have been found to be very important in causing human respiratory allergic disorders. Although the primary treatment for asthma and allergic rhinitis is essentially pharmacological [14], allergen avoidance should be attempted and desensitization with appropriate allergen should always be considered in chronic and severe cases. This measure, of course, requires knowledge about the sensitizing agents and pollen types. While antigenic and allergenic cross-reaction is a well-established fact [8, 5], skin and serum tests reveal that pollen allergic subjects are rarely sensitive to a single species; rather they are sensitive to a number of different pollen types at a time [10]. Since it has important implications for allergen avoidance and desensitization of pollen allergen, the present study was undertaken to establish the antigenic relation of those taxonomically related pollen types. The observation was based on cross-reaction as an aid to clinical management of the subjects, on the basis of IgG-ELISA and rocket immunoelectrophoresis using rabbit antisera.

Four major palm pollen types have been recorded from the air of Calcutta and suburbs, often in significant quantity and potent allergenicity [1, 2]. These are *Areca catechu* (areca palm), *Borassus flabellifer* (palmyra palm), *Cocos nucifera* (coconut palm) and *Phoenix sylvestris* (date sugar palm). The relevant palm trees are economically important in terms of yielding fruit, oil, sugar, liquors, etc., which grow naturally or are cultivated.

MATERIALS AND METHODS

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

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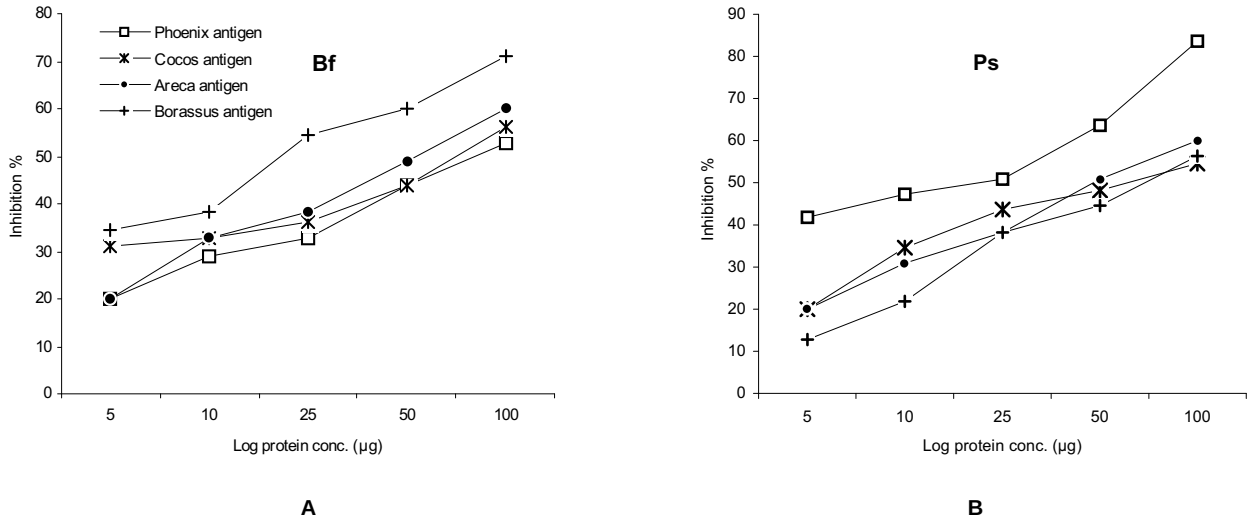


Figure 1. ELISA inhibition indicating the antigenic relationship among the four palm pollen allergens, Antibodies used are **A**) anti- *Borassus* and **B**) anti-*Phoenix* antibodies raised in rabbit. The solid phase used: Bf = *Borassus flabellifer* and Ps = *Phoenix sylvestris.* The binding of respective antibodies to the allergosorbents was inhibited with *Areca* , *Borassus* , *Cocos* and *Phoenix* antigen.

for 40 min. and the supernatant was brought to 90% saturation with ammonium-sulphate. The precipitate was dissolved in PBS and dialyzed (mol. wt. cut limit 10,000). The dialyzed extracts were filtered through 0.22 µm millipore filtre (Millipore, Bedford, Mass., 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* [7] using BSA as a standard.

Immunization for Rabbit Sera. Antiserum to *Phoenix sylvestris* and *Borassus flabellifer* were raised in rabbits. Each allergen extract (10 mg/ml) was mixed with an equal volume of Freund's complete adjuvant and the animals were administered with 1 ml of mixture intramuscularly for four consecutive weeks. Intramuscular booster injections with an emulsion of allergens with Freund's incomplete adjuvant were given after a two week interval. The animals were bled in the following week and the serum separated was collected and preserved in aliquots at -20°C until used.

ELISA Inhibition Using Anti IgG Enzyme Complex. ELISA inhibitions [9] were performed by incubating 100 µl of rabbit antisera with 100 µl of inhibitor (palm pollen extracts) in different concentrations overnight at 4°C. The inhibited serum was then added to the wells previously coated with antigen. Normal rabbit serum prior to the immunization with antigens were used as negative control. The antibodies bound to the plate were detected after overnight incubation by anti-rabbit IgG peroxidase enzyme labelled conjugate. Absorbance was measured at 492 nm. Percent inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{\text{O.D. of test sample - O.D. of inhibited sample}}{\text{O.D. of the test sample}} \times 100$$

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

Rocket Immunoelectrophoresis. Rocket immunoelectrophoresis was performed on a glass plate (5 × 7.5 cm) with 1% agarose gel containing rabbit antisera in Tris-barbital buffer (pH 8.6 containing 0.073 M Tris, 0.024 M Barbital and 0.003 M Na-azide) following the classical method of Laurell [6]. Ten µl (containing 50 µg protein) of each palm pollen extract was placed into the wells and the electrophoresis was run for 16 h (2 v/cm at 4°C). The slides were washed, pressed, dried and stained with Coomassie Brilliant Blue, R-250 (Sigma Chemical Co., USA).

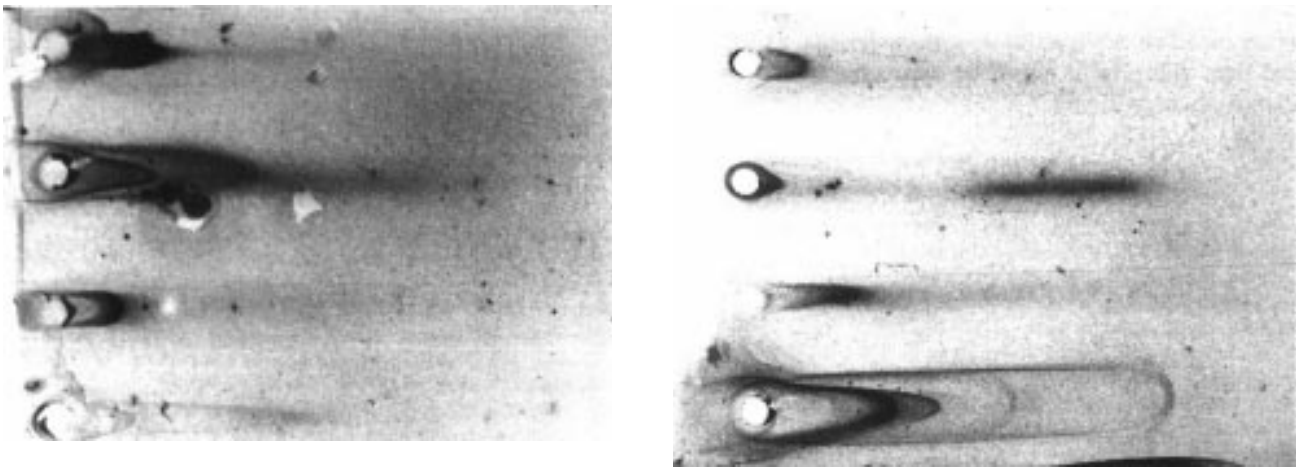
RESULTS

A clear cut inhibition of IgG binding, specific to the four palm pollen protein extracts, was obtained with comparable amounts of the extracts. Interestingly, it was also noted that not only the homologous extracts but also

Table 1. Antigenic relationship among the four palm pollen extracts as measured by IgG-ELISA inhibition with rabbit antisera

	Solid phase	Antigen used for inhibition*			
		Areca	*Borassus*	*Cocos*	*Phoenix*
Antigen	*Borassus*	58	21	75	82
	Phoenix	43	68	43	13

* Amount (µg) required for 50% inhibition.



A B

Figure 2. Rocket immunoelectrophoresis of the four palm pollen extracts using rabbit antisera against **A**) *Borassus* and **B**) *Phoenix* antigens. The antigens used in each lane from top to bottom were: *Areca, Borassus, Cocos* and *Phoenix.*

the heterologous extracts produced significant dose-dependent inhibition in the range of protein concentration (1-25 μg) tested (Fig. 1). Here, the antigenic relationship among the four palm pollen extracts was demonstrated by competitive ELISA inhibition using rabbit antibodies raised against *Borassus flabellifer* and *Phoenix sylvestris.* The percentage of inhibition was higher with the homologous antigen. *Phoenix*, *Cocos* and *Areca* gave an identical inhibition pattern when *Borassus* was used as solid phase antigen (Fig. 1a) and *Borassus* antibody was used. When *Phoenix* antibody was used, similarly (Fig. 1b), the other three, i.e., *Borassus*, *Cocos* and *Areca*, showed identical inhibition curve indicating the common relationship among them. The amount required for 50% inhibition is presented in Table 1. For *Borassus* antisera, the grade of inhibition in terms of C_{50} value gradually decreases with *Areca* > *Cocos* > *Phoenix* respectively. *Phoenix* antisera, *Areca* and *Cocos* had the same C_{50} value (43 μg), which was greater than *Borassus*.

In rocket immunoelectrophoresis, the anti-*Phoenix* antibody showed precipitation with all four palm pollen extracts, though the number of precipitin bands was more with homologous antibody (Fig. 2a). Also with anti-*Borassus* antibody (Fig. 2b), the other three extracts exhibited a good precipitation reaction.

DISCUSSION

Among the different types of allergenically potent airborne pollen, palm pollen grains were also found to be allergenic through aerobiological and clinical tests over different areas of Greater Calcutta [1, 2, 3, 4]. Here, the study was based on the idea that there may be some antigenic cross-reaction among the palm pollen grains due to the high probability of the presence of homologous proteins in the taxonomically related types [8, 13]. From India, antigenic and allergenic cross-reaction was reported in this context only for the grass family [12], whereas palm trees never drew attention, although these are important airborne components causing allergy. Antigenic cross-reaction was studied earlier for other grass pollen by using monoclonal antibody [11].

Here, antibodies were raised in rabbits against *Borassus* and *Phoenix* pollen protein extract. In rabbit IgG specific ELISA inhibition with anti-*Borassus* antibody, serial dilution of *Areca*, *Borassus* and *Cocos*, were able to inhibit its binding to solid phase *Borassus* antigen. In the case of *Phoenix* antibody similar results were also observed for the remaining three pollen types and the pattern of inhibition curves were very similar.

To confirm further and visualize directly the presence of antigenic cross-reactivity, rocket IEF was conducted with anti-*Borassus* and *Phoenix* rabbit sera in agarose gel. Both *Borassus* and *Phoenix* antigen showed a maximum number of precipitin rockets with homologous antisera respectively. The number of precipitin rockets were less with the other three pollen types, which may be due to lower antigenic recognition.

The C_{50} value, i.e., the amount of protein required for 50% ELISA inhibition, was studied using the relevant two antisera for all the types showing requirement of proteins in microquantities. For *Borassus* the maximum antigenic cross-reaction was showed by *Areca*, followed by *Cocos* and *Phoenix*. For *Phoenix* antisera, *Areca* and *Cocos* exhibited same level of cross-reaction.

These findings with palm pollen enhances the general thinking of the presence of shared antigens in the taxonomically-related pollen types.

CONCLUSION

Preliminary results indicate that the four relevant palm pollen grains, which are prevalent in the air of Calcutta and potent in causing respiratory allergy, have strong

cross-reactive antigenicity. The results prepare the basis of an detail in-depth study to detect the shared antigens and then allergens in terms of their applicability in the immunotherapy of pollen allergic asthmatics.

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