

## Analysis of IgE binding proteins of mesquite (*Prosopis juliflora*) pollen and cross-reactivity with predominant tree pollens

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### Abstract

Pollen from the mesquite tree, *Prosopis juliflora*, is an important source of respiratory allergy in tropical countries. Our aim was to partially characterize the IgE binding proteins of *P. juliflora* pollen extract and study cross-reactivity with prevalent tree pollen allergens. Intradermal tests with *P. juliflora* and five other tree pollen extracts were performed on respiratory allergy patients from Bikaner (arid) and Delhi (semi arid). *Prosopis* extract elicited positive skin reactions in 71/220 of the patients. Sera were collected from 38 of these 71 patients and all demonstrated elevated specific IgE to *P. juliflora*. Immunoblotting with pooled patients' sera demonstrated 16 IgE binding components, with components of 24, 26, 29, 31, 35, 52, 58, 66 and 95 kDa recognized by more than 80% of individual patients' sera. *P. juliflora* extract is allergenically potent requiring 73 ng of self-protein for 50% inhibition of IgE binding in ELISA inhibition. Cross-inhibition assays showed close relationship among *P. juliflora*, *Ailanthus excelsa*, *Cassia siamea* and *Salvadora persica*. IgE binding components of 14, 41, 52 and 66 kDa were shared allergens whereas 26 and 29 kDa were specific to *P. juliflora*. The findings suggest that purification of cross-reactive allergens will be helpful for diagnosis and immunotherapy of tree pollen allergic patients.

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**Keywords:** Allergens; Characterization; Cross-reactivity; *Prosopis juliflora*; Tree pollen

### Introduction

Pollen grains from diverse sources are known to induce type I hypersensitivity in predisposed individuals. Tropical countries like India are inhabited by a wide variety of plant species releasing large amounts of pollen

throughout the year (Singh et al., 1992a). Aerobiological and clinical studies detected more than 20 airborne pollen types responsible for respiratory allergies (Singh and Kumar, 2003). Grasses are the major contributors of pollen allergens followed by trees and weeds. Allergen extracts used for diagnosis and therapy are multi-allergenic systems containing mixtures of proteins and other components. It has been recommended to standardize extracts following IUIS/WHO protocols.

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However this could not be implemented due to a large number of extracts and scarcity of resources in different countries. The extracts are still standardized on w/v basis and skin tests, which does not express the true quality/quantity of individual components. Purified cross-reactive proteins are likely to reduce the number of allergens and thus overcome problems associated with standardizing whole pollen extracts (Weber, 2003).

Among the trees, *Prosopis juliflora* (mesquite) has been identified as an important source of pollen allergens (Abdulrahman et al., 1999; Arora and Jain, 2001; Killian and McMichael, 2004). Mesquite belongs to the family Mimosaceae and is originally from arid regions of Mexico and Central America. This has now acclimatized to various countries in Asia and the states of the Indian sub-continent. *Prosopis* flowers for almost the whole year (Feb–May; Aug–Nov) and incite sensitization in 6–11% of atopic cases as revealed by skin tests (Acharya, 1980; Singh and Kumar, 2003). Earlier, proteins of 66 and 59 kDa were identified as major allergens of *P. juliflora* pollen and firewood extract (More et al., 2002). But the detailed analysis of pollen allergens is required to identify major IgE binding components.

Previous studies have defined the allergenic properties of certain tree species namely *Holoptelea integrifolia*, *Betula verrucosa*, *Putranjiva roxburghii*, *Olea europea* and *Fraxinus excelsior* (Malik et al., 1991; Hirschwehr et al., 1993; Singh et al., 1997; Sanchez et al., 2002; Niederberger et al., 2002). Like grasses, tree pollens also contain some shared and specific allergens (Gonzalez et al., 2000; Niederberger et al., 2002). However, the knowledge about cross-reactive allergens of tree pollens prevalent in tropical countries is lacking. The present study was therefore aimed to partially characterize IgE binding components of *P. juliflora* pollen by immunoblot using a spectrum of hypersensitive patients' sera. For this purpose patients were recruited from two respiratory allergy clinics at S.P. Medical College, Bikaner and V.P. Chest Institute, Delhi. Also, the shared and specific allergens of *P. juliflora* with five predominant tree species were delineated by inhibition assays.

## Materials and methods

### Pollen samples

Polleniferous materials were collected from *P. juliflora* (mesquite) and five other tree species namely, *Ailanthus excelsa* (tree of heaven), *Cassia siamea* (pheasant wood), *Holoptelea integrifolia* (Indian elm), *Putranjiva roxburghii* (Indian amulet tree) and *Salvadora persica* (mustard tree) during peak flowering season. *C. siamea* belongs to

*Prosopis* family Mimosaceae and blooms from September–February. *A. excelsa* from Simarubaceae and *S. persica* from Salvadoraceae plant family, flower during November–January. *H. integrifolia* belonging to Ulmaceae and *P. roxburghii* to Euphorbiaceae produce enormous amount of pollen during March–May. Collection and processing of pollen material was done carefully by trained pollen collectors.

The inflorescences collected were frozen at  $-70^{\circ}\text{C}$  and freeze dried. Pollen grains were separated by pulverizing/passing the dried material through different sieves (100, 200 and 300 mesh) successively (Bijli et al., 2002). The fine powder thus obtained was subjected to a purity check for pollen content under a microscope. Pollen materials with >95% pollen and <5% floral parts of same plant were taken for antigen extraction.

### Preparation of extracts

Pollen materials were defatted using repeated changes of diethyl ether. The extracts were prepared in ammonium bicarbonate buffer (50 mM  $\text{NH}_4\text{HCO}_3$ , 1 mM PMSF and 2 mM EDTA) pH 8.0 by continuous stirring for 4 h at  $4^{\circ}\text{C}$ . The supernatant was separated by centrifugation at 10,000g for 30 min, filtered through 0.22  $\mu\text{m}$  membrane under sterile conditions and lyophilized. Protein concentration was determined by modified Lowry's method using phosphotungstic acid reagent for protein precipitation (Singh et al., 1992b).

### Carbohydrate estimation

Carbohydrate content of *P. juliflora* pollen was determined by phenol–sulphuric acid method (Dubois et al., 1956). Briefly, standard solution of D-glucose (5–30  $\mu\text{g}/\mu\text{l}$ ) was prepared and volume was made up to 1 ml with water. To the standard and sample (5–50  $\mu\text{g}$  *P. juliflora* extract in 1 ml water), 1 ml of 5% phenol was added, followed by 5 ml of concentrated  $\text{H}_2\text{SO}_4$  and vortexed. All the solutions were prepared in triplicates. The reaction mixture was incubated at room temperature for 30 min and absorbance of both standards/sample was read at 490 nm.

### Sodium dodecylsulphate polyacrylamide gel electrophoresis

Electrophoresis was carried out on 12% gel using discontinuous buffer system containing 0.025 M Tris, 0.2 M glycine and 0.1% SDS (Laemmli, 1970). One hundred microgram protein (pollen extract) was boiled for 5 min at  $100^{\circ}\text{C}$  in two fold concentration of sample buffer containing  $\beta$ -mercaptoethanol. The proteins were electrophoresed at a constant voltage of 120 V and bands were detected by staining with 0.1% Coomassie

brilliant blue R-250 or silver stained (Wayne et al., 1981).

### Selection of patients

A total of 220 patients suffering with allergic rhinitis, bronchial asthma and or both were included in the present study. These patients were recruited at two allergy clinics in S.P. Medical College, Bikaner ( $n = 117$ ) and V.P. Chest Institute, Delhi ( $n = 103$ ). The detailed history including age, sex, duration of illness, family history, onset of symptoms, seasonality etc. of each patient was recorded and clinical investigation was done with consent for allergy diagnosis. The Human Ethics Committee of the institute approved the study protocol.

### Skin testing

Intradermal tests were carried out on patients aged 18–46 years with *P. juliflora* pollen extract reconstituted in phosphate buffer saline (1:500 w/v in 0.1 M PBS). The patients were also skin tested with other tree pollen extracts namely *A. excelsa*, *C. siamea*, *P. roxburghii*, *S. persica* and *H. integrifolia*. Besides these tree pollens, grass and weed pollens, fungi and insect extracts procured from Antigen Laboratory, Institute of Genomics and Integrative Biology, Delhi were also tested. For skin tests, about 10  $\mu$ l of each extract was injected intradermally into the forearm of the patients. Histamine diphosphate 100  $\mu$ g/ml was injected as a positive control and PBS as a negative control. Skin tests were graded after 20 min based on wheal size in comparison to positive control. The reactions with wheal size equal to the size of positive control were marked as 2+. Skin reactions greater than positive control with two pseudopodia were graded as 3+ and with multiple pseudopodia as 4+ (Shanker et al., 2006).

Sera were collected for immunobiochemical assays from 38 patients with marked positive skin tests to *P. juliflora* extract. Sera were also collected from healthy volunteers (five from each center) and pooled to be used as negative control. For determination of cross-reactive proteins in *P. juliflora* with other tree pollen extracts, 5 separate serum pools (Table 1) were prepared with patients' sera skin test positive to *P. juliflora* but negative to pollen extracts being tested by immunological assays.

### Specific IgE estimation

Specific IgE levels against *P. juliflora* were determined by indirect ELISA in individual patients' sera. Briefly, 1  $\mu$ g protein in 100  $\mu$ l of carbonate-bicarbonate buffer (pH 9.6) was coated per well overnight at 4 °C. After washing the unbound antigen with PBS (0.1 M), free

**Table 1.** Skin test reactivity to *P. juliflora* in pollen sensitive patients

| Patient no. | Skin test positivity                             |
|-------------|--------------------------------------------------|
| 1           | PJ (+ +)                                         |
| 2           | PJ (+ +), HI (+), SP (+)                         |
| 3           | PJ (+ +), PR (+)                                 |
| 4           | PJ (+ +), AE (+ +)                               |
| 5           | PJ (+ +), CS (+ +)                               |
| 6           | PJ (+ +), HI (+), PR (+), CS (+), SP (+), AE (+) |
| 7           | PJ (+ +), HI (+), PR (+), CS (+), SP (+), AE (+) |
| 8           | PJ (+ +), PR (+), SP (+), HI (+)                 |
| 9           | PJ (+ +), CS (+) SP (+), HI (+)                  |
| 10          | PJ (+ +), PR (+), AE (+)                         |

PJ, *Prosopis juliflora*; AE, *Ailanthus excelsa*; CS, *Cassia siamea*; PR, *Putranjiva roxburghii*; SP, *Salvadora persica*; HI, *Holoptelea integrifolia*.

Serum pools used for cross-reactivity studies.

Pool a—serum from patients no. 1, 3, 4, 5, 10 used for immunoassay for HI/SP.

Pool b—serum from patients no. 1, 2, 4, 5, 9 used for immunoassay for PR.

Pool c—serum from patients no. 1, 2, 3, 5, 8, 9 used for immunoassay for AE.

Pool d—serum from patients no. 1, 2, 3, 4, 8, 10 used for immunoassay for CS.

Pool e—serum from patients no. 1 to 10 used for immunoassay for PJ.

sites were blocked with 3% defatted milk in PBS for 1 h at 37 °C. This was followed by three washings with PBS and overnight incubation with 100  $\mu$ l of 1:10 (v/v PBS) diluted individual patients' sera at 4 °C. The plate was washed five times with PBST (PBS with 0.05% Tween 20) and once with PBS. The wells were incubated with 1:1000 (v/v PBS) diluted polyclonal anti-human IgE peroxidase (Sigma, A-9667) for 3 h at 37 °C. The washing was carried out again with PBST followed by PBS and the color was developed using *O*-phenylenediamine in citrate-phosphate buffer pH 5.0. Reaction was stopped after 30 min with addition of 50  $\mu$ l of 5 N H<sub>2</sub>SO<sub>4</sub> and absorbance read at 492 nm. Normal human sera (NHS: a pool of five normal human sera) were used as a negative control. A non-relevant antigen (*Curvularia lunata* – fungus) was coated as control to check the non-specific binding.

### Specific IgE ELISA inhibition

To find out the potency of *P. juliflora* pollen extract, IgE binding was inhibited by pre-incubating hypersensitive patient's pooled sera (1:10 v/v in PBS) with 5, 10, 50, 100, 500, 1000, 5000 and 10,000 ng of self-extract (three batches Pj1, Pj2, Pj3) for 16 h at 4 °C. For

cross-reactivity study different tree pollen extracts (5–10,000 ng protein) were preincubated overnight at 4 °C with separate serum pools (1:10 v/v in PBS). The preincubated mixture was then added to microtitre plate coated with *Prosopis* extract and ELISA was performed. A pooled sera without an inhibitor was used as a positive control. Percentage inhibition was calculated as under:

$$1 - \frac{\text{OD of sample with inhibitor}}{\text{OD of sample without inhibitor}} \times 100.$$

In ELISA inhibition, an irrelevant extract of *C. lunata* was used as a control inhibitor.

### IgE Immunoblot

SDS-PAGE separated *P. juliflora* pollen proteins were transferred to nitrocellulose membrane in 25 mM Tris, pH 8.3 containing 192 mM glycine and 20% methanol (Bijli et al., 2002). Standard proteins of known molecular weight (Mw) (14–97 kDa; Bangalore genie, India) were also electrophoresed on the same gel along with sample proteins. Non-specific sites were blocked with 3% defatted milk in PBS for 1 h at 37 °C. The membrane was washed with PBS and incubated overnight at 4 °C with pooled and/or individual patients' sera (1:10 v/v in PBS) positive to *Prosopis* extract. As a control, a strip blotted with *Prosopis* pollen extract was incubated with pooled normal human serum (1:10 v/v in PBS). After washing five times with PBST and once with PBS, horseradish peroxidase labeled anti-human IgE (1:1000 v/v in PBS) was added to the blotted membrane and incubated for 3 h at 37 °C. The unbound antibodies were removed from blot by washing with PBST and PBS and blots were developed using 3'-3'-diamino benzidine and H<sub>2</sub>O<sub>2</sub> in 0.05 M sodium acetate buffer (pH 5). The blots were washed after 5 min with distilled water to stop the color reaction.

Pollen extracts of five dominant tree species were also transferred onto nitrocellulose membrane and incubated with different serum pools positive to *P. juliflora* but negative to the other extract bound on the membrane. One *P. juliflora* strip was probed with NHS and another strip containing *C. lunata* was probed with hypersensitive pooled patients' sera.

### IgE immunoblot inhibition

SDS-PAGE resolved *P. juliflora* proteins were transferred to nitrocellulose membrane. After blocking the free sites, strips were kept overnight at 4 °C with a preincubated mix of 500 µl of patients' sera (1:5 v/v) and 500 µl of *P. juliflora* protein extract (200 µg) and other tree pollen species. As positive control, a *P. juliflora* strip was incubated with hypersensitive pooled patients' sera (1:10 v/v). Another *P. juliflora* strip probed with NHS

served as a negative control. Strips were washed three times with PBST, incubated with anti-human IgE peroxidase (1:1000 v/v) for 3 h at 37 °C and blots developed.

## Results

The protein content in three batches of *P. juliflora* pollen extract was 70, 76 and 78 mg/g of pollen. The protein to carbohydrate ratio (P:C) was 1:0.64 in pollen extract of *P. juliflora*. SDS-PAGE resolved pollen extract into 33 distinct protein bands ranging from 14 to 101 kDa. Three batches of pollen extract demonstrated same number of bands on SDS-PAGE (Fig. 1). Intradermal tests showed strong positive reactivity to *P. juliflora* pollen extract in 60 (51.3%) patients from Bikaner and 11 (10%) from Delhi region. Specific IgE values were determined against PJ by ELISA in 38 individual patients' sera collected from Bikaner (28) and Delhi (10). The patients from both the centers showed significantly elevated IgE levels, three times or more of the normal control (NHS O.D. = 0.078 Bikaner; 0.105 Delhi). Specific IgE values in Bikaner patients ranged from O.D. 0.242 to 0.793 (mean ± SD = 0.416 ± 0.132) whereas in patients from Delhi, it ranged from 0.312 to 0.678 (mean ± SD = 0.422 ± 0.123).

ELISA inhibition was carried out to determine the potency of three different batches (Pj1, Pj2 and Pj3) of pollen extract with similar SDS-PAGE profile. For 50% inhibition of IgE binding to *Prosopis* extract bound to solid phase, 80, 75 and 65 ng of self-protein was required from three batches of *P. juliflora* pollen extract (Fig. 2).

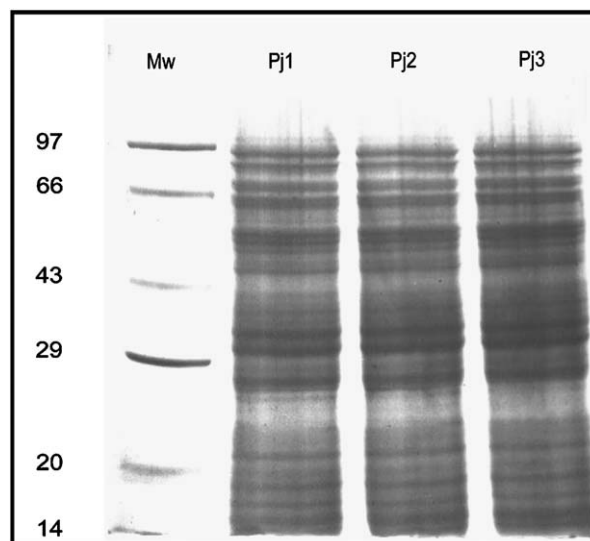


**Fig. 1.** SDS-PAGE profile of *Prosopis juliflora* pollen extract. Pj1, Pj2 and Pj3 are three different batches of extract. Approximately 100 µg protein was loaded and bands were visualized with Coomassie stain. Mw: Molecular weight markers.

Immunoblotting of *P. juliflora* pollen extract with pooled patients' sera detected 16 IgE binding components of 14, 16, 20, 24, 26, 29, 31, 35, 45, 41, 52, 58, 66, 72, 95 and 97 kDa (Fig. 3A and B). Proteins of 24, 26, 29, 31, 35, 52, 58, 66 and 95 kDa were recognized by more than 80% of individual patients' sera, hence considered as major IgE binding components in *P. juliflora* extract. On separate analysis of Bikaner and Delhi patients, protein components of 26, 29, 52, and 66 kDa were detected as common major allergens at both centers.



**Fig. 2.** IgE ELISA inhibition of *Prosopis juliflora* pollen extract. *Prosopis* extract 1 µg/well, was coated and incubated with a mixture containing *Prosopis* pooled sera (1:10) and 5, 10, 50, 100, 500, 1000, 5000 and 10,000 ng of extract as inhibitors (□ PJ1, ♦ PJ2, and △ PJ3). The bound IgE was determined using anti-human IgE-HRP. The values represent the mean of three independent experiments.

To identify the cross-reactivity of *P. juliflora* extract, ELISA inhibition was carried out with serum pool (Table 1) of patients hypersensitive to *P. juliflora* using *A. excelsa*, *C. siamea*, *P. roxburghii*, *S. persica* and *H. integrifolia* as inhibitors. *P. juliflora* extract required approximately 70 ng of self-protein for 50% inhibition whereas *A. excelsa*, *C. siamea*, *P. roxburghii*, *S. persica* and *H. integrifolia* produced same inhibition at 110, 125, 125, 160 and 200 ng, respectively (Fig. 4). *C. lunata*, a non-relevant extract however could not cause 50% inhibition in *P. juliflora* extract even at 10 µg concentration. For self-inhibition (50%) of specific IgE binding, these tree species required 65–80 ng of protein(s).

IgE binding proteins common in *P. juliflora* and the above tree pollen extracts were studied using four separate serum pools as described in Table 1. IgE immunoblots of different pollen extracts showed 7–15 allergenic bands (Fig. 5A). Nine IgE binding components of 14, 16, 20, 35, 41, 52, 58, 66 and 95 kDa in *A. excelsa* were similar in Mw to allergenic components of *P. juliflora*, followed by 8 each in *C. siamea* and *S. persica*. *P. roxburghii* and *H. integrifolia* however, showed 5 IgE reactive components similar to *P. juliflora* (Table 2).

To confirm the cross-reactivity of *P. juliflora* with tree pollen allergens, immunoblot inhibition was performed using different serum pools (Table 1) and respective tree pollen extract as inhibitor. SDS-PAGE separated *P. juliflora* proteins were transferred to nitrocellulose

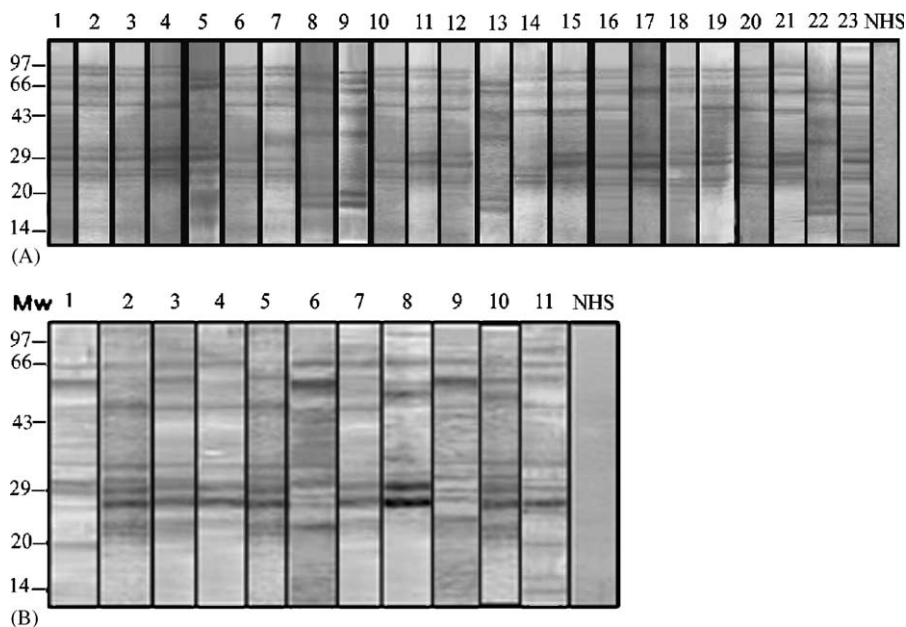


**Fig. 3.** (A) IgE immunoblot of *Prosopis juliflora* pollen extract using hypersensitive patients' sera (1:10) from Bikaner. Lane 1 to 22: Probed with individual patient's serum; Lane 23: Probed with pooled sera; NHS: probed with pooled normal human sera; Mw: Molecular weight markers. (B) IgE immunoblot of *Prosopis* extract using hypersensitive patients' sera (1:10) from Delhi. Lane 1 to 10: Probed with individual patient's serum; Lane 11: Probed with pooled sera; NHS: probed with pooled normal human sera; Mw: Molecular weight markers.

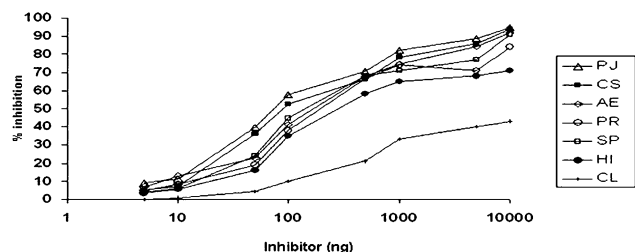


**Fig. 4.** IgE ELISA inhibition with tree pollen extracts. *Prosopis* extract 1  $\mu$ g/well, was coated and incubated with a mixture containing *Prosopis* pooled sera (1:10) and 5, 10, 50, 100, 500, 1000, 5000 and 10,000 ng of extract as inhibitors ( $\Delta$  PJ; *P. juliflora*,  $\blacksquare$  CS; *C. siamea*,  $\diamond$  AE; *A. excelsa*,  $\circ$  PR; *P. roxburghii*,  $\square$  SP; *S. persica*,  $\bullet$  HI; *H. integrifolia* and + CL; *C. lunata*). The bound IgE was determined using anti-human IgE-HRP. The values represent the mean of three independent experiments.

membrane and then incubated with the inhibited serum pool. *P. juliflora* extract showed complete inhibition of specific IgE reactivity at 200  $\mu$ g of self-protein (Fig. 5B). For cross-inhibition, maximum numbers of bands in *P. juliflora* were inhibited by *C. siamea* and *S. persica* followed by *A. excelsa* used as inhibitor. Of the four major allergenic proteins 26, 29, 52 and 66 kDa of *P. juliflora*, proteins of 52 and 66 kDa were completely inhibited by all the five tree pollen extracts, indicating shared IgE epitopes (Fig. 5B). Proteins of 26 and 29 kDa were however, not inhibited by any of the tree pollen extract tested and therefore can be considered as specific allergens of *P. juliflora*.

## Discussion

*P. juliflora* is a significant contributor of pollen in tropical and subtropical regions of the world. There are a few reports on allergenicity to *P. juliflora* from America, India, Kuwait and United Arab Emirates (Novey et al., 1977; Shivpuri, 1980; Thakur and Sharma, 1985; Abdulrahman et al., 1999; Lestringant et al., 1999). In the present study, *P. juliflora* showed sensitization by skin tests in 60/117 (51.3%) patients at Bikaner (arid region) as compared to 11/103 (10%) patients at Delhi (semi arid). Higher skin test positivity in Bikaner may be due to massive exposure of subjects to a major single tree allergen. Bikaner lies in the Thar desert region where the climate is warm and dry, suitable for *P. juliflora*, and therefore it is a dominant species, whereas in Delhi, the atopics are exposed to multiple pollen allergens causing polysensitization. Specific IgE levels of patients ( $n = 38$ ) from both the regions were significantly elevated, compared to normal control. An earlier study from Kuwait also showed varied skin

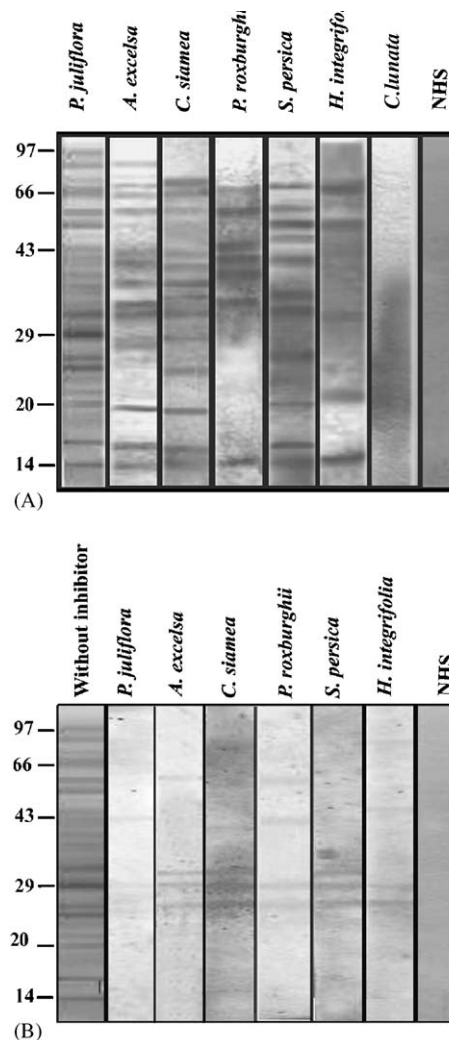


**Fig. 5.** (A) Immunoblot of *P. juliflora* and five tree pollen extracts showing IgE reactive bands. Pollen extracts were probed with serum pools from *P. juliflora* sensitive patients as described in Table 1. NHS: probed with pooled normal human sera; C: strip containing *C. lunata* as an irrelevant control; Mw: Molecular weight markers. (B) IgE immunoblot inhibition with tree pollen extracts. *P. juliflora* protein strips were incubated with serum pools containing 200  $\mu$ g of different tree pollen extract as inhibitors. NHS: probed with pooled normal human sera; Mw: Molecular weight markers.

reactivity to *Prosopis*, ranging from 11% to 76% in patients from four different geographical regions (Abdulrahman et al., 1999).

The allergenic potency of *P. juliflora* pollen extract was evaluated by ELISA inhibition. It showed a dose dependent response with 65–80 ng of self-protein required for 50% inhibition of IgE binding. SDS-PAGE separated *P. juliflora* pollen extract showed 16 IgE binding components of 14, 16, 20, 24, 26, 29, 31, 35, 41, 45, 52, 58, 66, 72, 95 and 97 kDa. In a recent study, 13 allergenic components of 11, 16, 17, 18, 20, 27, 30, 36,

**Table 2.** Shared IgE binding bands recognized in different pollen extracts using *P. juliflora* hypersensitive pooled patients' sera

| Pollen                         | No. of protein bands | No. of allergenic proteins | Shared allergenic proteins (kDa)                                                           |
|--------------------------------|----------------------|----------------------------|--------------------------------------------------------------------------------------------|
| <i>Prosopis juliflora</i>      | 33                   | 16                         | <b>14</b> , 16, 20, 24, 31, <b>35</b> , <b>41</b> , 48, <b>52</b> , 58, <b>66</b> , 72, 95 |
| <i>Ailanthus excelsa</i>       | 22                   | 15                         | <b>14</b> , 16, 20, <b>35</b> , <b>41</b> , <b>52</b> , 58, <b>66</b> , 95                 |
| <i>Cassia siamea</i>           | 23                   | 15                         | <b>14</b> , 16, 20, <b>35</b> , <b>41</b> , <b>52</b> , <b>66</b> , 75                     |
| <i>Putranjiva roxburghii</i>   | 20                   | 12                         | <b>14</b> , <b>35</b> , <b>41</b> , <b>52</b> , <b>66</b>                                  |
| <i>Salvadora persica</i>       | 17                   | 12                         | <b>14</b> , 16, 20, 31, <b>35</b> , <b>41</b> , <b>52</b> , <b>66</b>                      |
| <i>Holoptelea integrifolia</i> | 15                   | 8                          | <b>14</b> , <b>35</b> , <b>41</b> , <b>52</b> , <b>66</b>                                  |

44, 56, 71, and 99 kDa were identified in *P. juliflora* pollen extract (Killian and McMichael, 2004). There were minor difference in Mws of proteins recognized in present study and those demonstrated by Killian and McMichael (2004). These differences may be due to variation in the pollen extracts, serum samples and the method used for calculating the Mw of protein components (Killian and McMichael, 2004). In the present study, immunoblot analysis with individual patient's serum demonstrated diverse IgE reactivity with nine proteins of 24, 26, 29, 31, 35, 52, 58, 66 and 95 kDa as major IgE binding components. The protein of 66 kDa was recognized by 90% of the patients' sera tested. In a previous study, 59 and 66 kDa *P. juliflora* pollen proteins demonstrated IgE reactivity with SPT positive patients' sera (More et al., 2002). These proteins were also present in Mesquite wood extract and the smoke collected after burning the wood.

The knowledge of pollen cross-reactivity is crucial for diagnostics as well as formulation of immunotherapy vaccines. Cross-reactivity among pollens belonging to same genus and/or different genera has been demonstrated earlier (Pham and Baldo, 1995; Weber, 2003; Sastre et al., 2004). Proteins of 15, 18, 19, 20, 40–46, 60 and 70 kDa have been shown cross-reactive among trees and other plant species (Lombardero et al., 2002; Schwietz et al., 2000; Heiss et al., 1996; Gruehn et al., 2003). Some cross-reactive proteins were genetically engineered and are found to have potential for use in immunotherapy (Niederberger et al., 2004; Westritsch-nig et al., 2004). In the present study, cross-reactivity of *P. juliflora* was investigated with five tree species dominant in Asia, namely *A. excelsa*, *H. integrifolia*, *C. siamea*, *P. roxburghii* and *S. persica*. *Salvadora* and *Prosopis* species are also distributed to arid (desert) regions in other continents. The tree species selected for this study are well known sources of allergens (Malik et al., 1991; Singh et al., 1997; Singh and Kumar, 2003). IgE inhibition assays demonstrated cross-reactive proteins of 14, 16, 35, 41, 52 and 66 kDa in *P. juliflora* and these tree species. The major IgE binding components of 52 and 66 kDa in *P. juliflora* extract were inhibited by all tree pollen extracts. However, 26 and 29 kDa protein components were specific to *P. juliflora*.

In conclusion, *P. juliflora* pollen is a potent allergen source with four major IgE binding components. It shows a close allergenic relationship with *A. excelsa*, *C. siamea* and *S. persica* tree species. The observations suggest that 52 and 66 kDa proteins have potential to be used as diagnostic and therapeutic reagent for patients allergic to mesquite and allergenically related tree species.

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## References

- Abdulrahman, A., Syed, M.H., Mohammed, O.G., Turki, A., Khalid, A., Sultan, T.A., 1999. Human sensitization to *Prosopis juliflora* antigen in Saudi Arabia. *Ann. Saudi Med.* 19, 331–336.
- Acharya, P.J., 1980. Skin test response to some inhalant allergens in patient's of nasobronchial allergy from Andhra Pradesh. *Aspect Allergy Appl. Immunol.* 13, 14–18.
- Arora, A., Jain, V.K., 2001. Aeropalynological survey at Bikaner. *Ind. J. Aerobiol.* 14, 28–32.
- Bijli, K.M., Singh, B.P., Sridhara, S., Gaur, S.N., Arora, N., 2002. Standardizing *Imperata cylindrica*—source material for quality allergen preparations. *J. Immunol. Methods* 260, 91–96.
- Dubois, M., Giles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- Gonzalez, E.M., Villalba, M., Rodriguez, R., 2000. Allergenic cross-reactivity of Olive pollen. *Allergy* 55, 658–663.
- Gruehn, S., Suphioglu, C., O'Heir, R.E., Volkmann, D., 2003. Molecular cloning and characterization of Hazel Pollen protein (70 kD) as a luminal binding protein (BiP): a novel cross-reactive plant allergen. *Int. Arch. Allergy Immunol.* 131, 91–100.

- Heiss, S., Fischer, S., Muller, W.D., Weber, B., Hirschwehr, R., Spitzauer, S., Kraft, D., Valenta, R., 1996. Identification of a 60 kD cross-reactive allergen in pollen and plant derived food. *J. Allergy Clin. Immunol.* 98, 938–947.
- Hirschwehr, R., Jager, S., Horak, F., Ferreira, F., Valenta, R., Ebner, C., Kraft, D., Scheiner, O., 1993. Allergens from birch pollen and pollen of the European chestnut common epitopes. *Clin. Exp. Allergy* 23, 755–761.
- Killian, S., McMichael, J., 2004. The human allergens of mesquite (*Prosopis juliflora*). *Clin. Mol. Allergy* 2, 8.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685.
- Lestringant, G.G., Bener, A., Frossard, P.M., Abdulkhalik, S., Bouix, G., 1999. A clinical study of airborne allergens in the United Arab Emirates. *Allergol. Immunol. (Paris)* 31, 263–267.
- Lombardero, M., Obispo, T., Calabozo, B., Leazaun, A., Barber, D., 2002. Cross-reactivity between olive and other species. Role of Ole 1-related proteins. *Allergy* 57 (Suppl 71), 29–34.
- Malik, P., Singh, A.B., Gangal, S.V., Babu, C.R., 1991. Comparison of antigenic and allergenic components of *Holoptelia integrifolia* pollen collected from different source materials. *Allergy* 46, 284–291.
- More, D., Whisman, L., Whisman, B., Jordan-Wagner, D., 2002. Identification of specific IgE to mesquite wood smoke in individuals with mesquite pollen allergy. *J. Allergy Clin. Immunol.* 110, 814–816.
- Niederberger, V., Purohit, A., Oster, J.P., Spitzauer, S., Valenta, R., Pauli, G., 2002. The allergen profile of ash (*Fraxinus excelsior*) pollen: cross-reactivity with allergens from different plant species. *Clin. Exp. Allergy* 32, 933–941.
- Niederberger, V., Horak, F., Vrtala, S., Spitzauer, S., Krauth, M.T., Valent, P., Reisinger, J., Pelzmann, M., Hayek, B., Kronqvist, M., Gafvelin, G., Gronlund, H., Purohit, A., Suck, R., Fiebig, H., Cromwell, O., Pauli, G., van Hage-Hamsten, M., Valenta, R., 2004. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc. Natl. Acad. Sci. USA* 101 (Suppl. 2), 14677–14682.
- Novey, H.S., Roth, M., Wells, I.D., 1977. Mesquite pollen – an aeroallergen in asthma and rhinitis. *J. Allergy Clin. Immunol.* 59, 359–363.
- Pham, N.H., Baldo, B.A., 1995. Allergenic relationship between taxonomically diverse pollens. *Clin. Exp. Allergy* 25, 599–606.
- Sanchez, J.C., Iraola, V.M., Sastre, J., Florido, F., Boluda, L., Fernandez-Caldas, E., 2002. Allergenicity and immunochemical characterization of six varieties of *Olea europea*. *Allergy* 57, 313–318.
- Sastre, J., Lluch-Bernal, M., Bustillo, A.M., Carnes, J., Maranon, F., Casanovas, M., Fernandez-Caldas, E., 2004. Allergenicity and cross-reactivity of Russian olive pollen (*Eleagnus angustifolia*). *Allergy* 59, 1181–1186.
- Schwietz, L.A., Goetz, D.W., Whisman, B.A., Reid, M.J., 2000. Cross-reactivity among conifer pollens. *Ann. Allergy Asthma Immunol.* 84, 87–93.
- Shanker, J., Singh, B.P., Gaur, S.N., Arora, N., 2006. Recombinant glutathione-S-transferase a major allergen from *Alternaria alternata* for clinical use in allergy patients. *Mol. Immunol.* 43, 1927–1932.
- Shivpuri, D.N., 1980. Clinically important pollen, fungal and insect allergens for nasobronchial allergy patients in India. *Aspect. Allergy Appl. Immunol.* 13, 19–23.
- Singh, A.B., Kumar, P., 2003. Aeroallergens in clinical practice of allergy in India. An overview. *Ann. Agric. Environ. Med.* 10, 131–136.
- Singh, B.P., Singh, A.B., Gangal, S.V., 1992a. Pollen calendars of different states, India. CSIR centre for Biochemicals, Delhi and National Botanic Research Institute, Lucknow, pp.3–23.
- Singh, B.P., Sridhara, S., Arora, N., Gangal, S.V., 1992b. Evaluation of protein assay methods for pollen and fungal spore extracts. *Biochem. Int.* 27, 477–484.
- Singh, B.P., Verma, J., Sridhara, S., Rai, D., Rai, D., Makhija, N., Gaur, S.N., Gangal, S.V., 1997. Immunobiochemical characterization of *Putranjiva roxburghii* pollen extract and cross-reactivity with *Ricinus communis*. *Int. Arch. Allergy Immunol.* 114, 251–257.
- Thakur, I.S., Sharma, J.D., 1985. Isolation and characterization of allergens of *Prosopis juliflora* pollen grains. *Biochem. Int.* 1, 903–912.
- Wayne, W., Teni, B., Virginia, P.W., Ronald, H., 1981. Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* 118, 197–203.
- Weber, R.W., 2003. Patterns of pollen cross allergenicity. *J. Allergy Clin. Immunol.* 112, 229–239.
- Westritschnig, K., Focke, M., Verdino, P., Goessler, W., Keller, W., Twardosz, A., Mari, A., Horak, F., Wiedermann, U., Hartl, A., Thalhamer, J., Sperr, W.R., Valent, P., Valenta, R., 2004. Generation of an allergy vaccine by disruption of the three-dimensional structure of the cross-reactive calcium-binding allergen, Phl p 7. *J. Immunol.* 172, 5684–5692.