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Several aerobiological and clinical studies have identified the pollen of *Pennisetum typhoides* as one of the main triggers of grass pollen induced type 1 allergic diseases in India. Its crude extract is currently being used in allergy clinics of India for the diagnosis and immunotherapy of grass pollen induced allergies. Since crude extract are known to carry a number of allergenic and non-allergenic components, it is essential to characterize the allergenic extract of *Pennisetum typhoides* pollen for the better management of allergic diseases. In the present study, this pollen extract has been characterized with respect to its various immunobiochemical properties.

A highly allergenic whole pollen extract of *Pennisetum typhoides* pollen was prepared in 0.05 M ammonium bicarbonate buffer. On an average, 1 gram dried and defatted pollen was found to contain 71.33 mg proteins and 28.07 mg carbohydrates as estimated by the method of Lowry et al. (1951) and Dubois et al. (1956), respectively. Out of 66 grass pollen allergic patients, 26 showed markedly positive skin sensitivity to the pollen extract of *Pennisetum typhoides*. Serum samples were collected from these patients. Elevated levels of specific IgE against components of *Pennisetum* pollen extract were estimated by ELISA. ELISA-inhibition with pooled patient sera demonstrated the highly potent nature of *Pennisetum* pollen extract prepared in this study, as 280 ng of it was sufficient to produce 50% inhibition of binding of specific IgE antibodies to solid phase pollen extract. Thirty seven silver stained protein bands were observed in the acidic pl zone, when crude whole pollen extract was subjected to TLIEF. Similarly on SDS-PAGE, 24-26 CBB stained bands in the molecular weight range of 10-100 kDa were observed. Of these 12 proteins in the molecular weight range of 14-85 kDa were identified as allergenic by immunoblot with pooled patients sera. Proteins with molecular weight 85, 80 and 30 kDa were detected as glycosylated. Specificity of the allergenic proteins was evident in the immunoblot inhibition assay. Upto 12 antigenic proteins were identified by immunoblot analysis with *Pennisetum* specific rabbit anti-sera. Proteins with molecular weight 70, 50, 30 and 14 kDa were found to be highly antigenic. Three different batches of pollen extract were studied and no batch to batch variation was
observed. Reference immunobiochemical characteristics were thus generated in the present study.

Because of the heterogeneous nature of immune response of different patients against components of crude pollen extract, it is imperative to identify all potential allergens present in the extract. Therefore, a full allergenic profile of *Pennisetum* pollen extract was studied by carrying out immunoblot with 26 individual patient sera. In all 12 allergens were identified in the molecular weight range of 14-85 kDa. Of these, 8 allergenic proteins with molecular weight 85, 80, 70, 50, 53, 40 and 34 kDa were identified as major allergens. Three major allergens with molecular weight 85, 70 and 43 kDa were recognized by 96.15% of 26 patient sera tested.

In order to purify the most potent major allergen from the crude whole pollen extract, a combination of DEAE Sephaex A50 and Sephadex G100 was used. ELISA inhibition was performed to identify the most allergenic fraction obtained from different columns. Ten different fractions were eluted when 90% ammonium sulfate precipitated pollen extract was loaded on a DEAE sephadex A50 column. Fraction Pen 5 was found to be the most allergenic fraction by ELISA-inhibition assay. Further fractionation of Pen 5 over sephadex G 100 column resulted in four different subfractions. Of these, subfraction Pen 5d was identified as most potent allergenic subfraction as it competed closely with crude pollen extract in ELISA inhibition assay. HPLC analysis demonstrated it to be a homogeneous protein. SDS-PAGE (under both, reducing as well as non-reducing condition) and TLEIF showed it to be a single polypeptide of 43 kDa size with pl 3.7, thereby confirming the purity of Pen 5d. No trace of glycosylation was found by two different methods namely phenol-sulphuric acid and DIG based immunoblot detection method. N-terminal of Pen 5d was found to be blocked to Edman degradation reaction. This protein was identified by patient sera from a test population of 64 grass allergic patients by dot blot. Comparable specific IgE levels against purified allergen and crude pollen extract were estimated by ELISA. The purified allergen was named as Pen 1 t 1, as per the recommendations of IUIS/WHO allergen nomenclature sub-committee. It was found to be highly antigenic in rabbits as judged by ELISA inhibition against crude pollen extract.

A number of grass species have been identified as allergenic pollen producers. It is cumbersome to study the allergenic nature of each and every grass
and include in the testing kit for the diagnosis of type I allergies. Studies with grasses in Europe, Australia and the USA have shown extensive cross-reactivity among different grass pollen allergens. However, not much is known about the cross reactive nature of allergens present in *Pennisetum typhoides*. Eight different grasses were studied by ELISA inhibition and Immunoblot analysis with pooled patients sera and *Pennisetum* specific rabbit anti-sera. A number of IgE binding components were detected in each of the grass pollen extract with *Pennisetum* specific pooled patients sera. Poa, Lolium, *Pennisetum*, Phleum, Sorghum, Cenchrus, Cynodon and Imperata extract revealed 14, 13, 12, 11, 9, 8, 7 and 5 IgE binding components, respectively. IgE binding bands with molecular weights 57, 43, and 30 kDa were observed in all grasses. These results indicate that these three allergens either represent a set of common allergens or proteins with shared allergenic epitopes on them, present in different grass pollens. Fourteen IgG binding bands were observed in the *Poa pratensis* and *Phleum pratense* pollen extracts when Immunoblot was carried out with *Pennisetum* specific rabbit anti-sera. Cynodon dactylon pollen extract showed the presence of only two weak antigenic bands. *Pennisetum typhoides*, *Lolium perenne*, Sorghum vulgare, Cenchrus ciliaris and Imperata cylindrica pollen extracts showed 12, 12, 12, 6 and 8 IgG binding components, respectively, in the molecular weight range of 14-85 kDa. Antigenic bands with molecular weights 70, 50, 43, 34 and 14 kDa were detected in 6 out of 8 grass pollen extracts.

Monoclonal antibody IG 12 identified group I allergens in pollen extracts of *Phleum pratense*, *Lolium perenne*, *Poa pratensis* and *Pennisetum typhoides* when eight different pollen extracts were screened by Immunoblot. On the other hand, Bo1 identified Lol p 5, Poa p 5 and Phl p 5 allergens from the pollen extracts of only *Lolium perenne*, *Poa pratensis* and *Phleum pratense*, respectively.

Except Cynodon, all heterologous grass pollen extracts produced significant inhibition of specific IgE (from pooled patients sera) binding to solid phase *Pennisetum typhoides* pollen extract. The binding of IgE to solid phase *Pennisetum typhoides* pollen extract was substantially inhibited by Poa and Sorghum extracts for which ID<sub>50</sub> was estimated as 280 and 285 ng, respectively, less than the ID<sub>50</sub> value obtained with homologous inhibitory pollen extract (i.e. *Pennisetum typhoides*). This suggests that Poa and Sorghum extract carry most of the allergenic epitopes present in *Pennisetum* extract. Antigenic relationship among these grasses was also
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evaluated by competitive ELISA inhibition by using *Pennisetum typhoides* specific rabbit anti-sera. Except *Cynodon* all other grass pollen extracts showed close antigenic relationship as they produced similar lines of specific IgG inhibition.

In conclusion, a set of reference immunobiochemical characteristics have been generated which would be beneficial in the quality control of the *Pennisetum typhoides* pollen extract as well as in the future standardization of this pollen extract. It has been shown that pollen extract of *Pennisetum* carries upto 12 allergenic proteins. *Pennisetum typhoides* pollen extract has been found to possess unique as well as shared allergens/antigens. A highly potent allergen Pen t 1 has been purified and characterized immunobiochemically. Identification of allergenic epitopes on Pen t 1 would be helpful in designing peptides based new therapeutics for the treatment of grass pollen induced allergic diseases.