

Airborne load of *Cassia* pollen in West Bengal, eastern India: its atmospheric variation and health impact

Mir Musaraf Hussain · Jyotshna Mandal ·
Kashinath Bhattacharya

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Abstract A Burkard personal volumetric sampler was used at Sriniketan, a town about 150 km northwest of Calcutta, in the state of West Bengal, in eastern India to record the frequency of three common airborne *Cassia* pollen types, *Cassia tora*, *Cassia occidentalis*, and *Cassia fistula* for two consecutive years (2004–2006). Correlation was made between the meteorological factors and the pollen concentration in the atmosphere. The study reports *Cassia* pollinosis by in vivo skin prick test in respiratory allergic patients. The highest positive reactions were exhibited by *C. tora* (34.7 %), *C. fistula* (33.3 %), and *C. occidentalis* (28.5 %). The allergic potential of these was investigated by in vitro enzyme linked immunosorbent assay test. Their protein components were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, in the range of 15.8–81.5 kDa. In *C. occidentalis* and *C. fistula*, 11 bands were found, while it was 10 in *C.*

tora. The results show that the *Cassia* pollen occur significantly in the atmosphere with the potential to elicit an allergic response in susceptible patients.

Keywords Aerobiology · Allergy · *Cassia* pollen · Skin prick test

Introduction

The worldwide prevalence and increase in asthma and allergic rhinitis has enhanced the monitoring for allergenic pollen grains (Kosisky et al. 2011). Regional aeroallergen monitoring gives useful information for the respiratory allergic patients as well as the clinicians. It has been estimated that more than 10 % of the Indian population suffer from major allergic or upper respiratory tract diseases and majority of these are from pollen grains (Boral and Bhattacharya 2000; Boral et al. 1999, 2004; Chowdhury et al. 1998; Sharma et al. 2006; Singh and Singh 1994; Singh and Sahi 2008). Airborne pollen grains are well-known cause and potent source of aeroallergens in India and elsewhere (Celenk et al. 2010; Mandal et al. 2011).

Calcutta, capital of the state of West Bengal, is India's third most populous city with a population of more than 15 million (e-CENSUS India 2011). Various aerobiological studies undertaken in West Bengal have revealed several pollen grains causing pollinosis such as pollen from the Poaceae family, *Azadirachta indica*, *Alstonia scholaris*, *Areca catechu*, *Cocos nucifera*, *Carica*

M. M. Hussain · K. Bhattacharya (✉)
Department of Botany, Visva-Bharati University,
Santiniketan 731235, West Bengal, India
e-mail: kashinathb23@rediffmail.com

J. Mandal
Division of Plant Biology, Bose Institute,
93/1 Acharya Prafulla Chandra Road,
Calcutta 700 009, India

Present Address:

J. Mandal
Institute of Evolutionary Biology and Environmental
Studies, University of Zurich,
Winterthurerstrasse 190,
8057, Zurich, Switzerland

papaya, *Delonix regia*, *Peltophorum pterocarpum*, and several others (Boral et al. 2004; Chakraborty et al. 1998; Chakraborty et al. 2009; Hussain et al. 2012; Mandal et al. 2008, 2011).

Cassia sp. of the family Fabaceae is widely distributed in the tropics and subtropics. Among the various species, *Cassia fistula*, *Cassia occidentalis*, and *Cassia tora* are very common in the Indian subcontinent especially in West Bengal (Mondal et al. 1998). *C. fistula* or the Golden shower tree is a flowering plant, native to southern Asia. *C. occidentalis* is an erect pan tropical annual herb. *C. tora* is also an annual and a tropical herb.

Cassia pollen grains have been reported in the atmosphere in various aerobiological surveys conducted in West Bengal (Calcutta and its suburbs) (Banik and Chanda 1990, 1992; Boral et al. 2004; Chakraborty et al. 1998; Mandal et al. 2006, 2008). Previous reports of its atmospheric presence have been made from northern India (Anonymous 2000; Ahlawat et al. 2010; Chauhan and Goyal 2006; Sahney and Chaurasia 2008), central India (Tripathi et al. 2004), southern India (Agashe and Anand 1982; Satheesh et al. 1994), and northeast India (Sharma et al. 2010). Other species of *Cassia* pollen (*Cassia siamea* and *Cassia alata*) have been reported to cause sensitization in respiratory allergic patients by other workers in 5 to 46 % of atopic cases, as revealed by skin prick test (Boral et al. 2004; Chauhan and Goyal 2006; Parui et al. 2002; Sharma et al. 2009).

The objective of the present work is (1) to determine the concentration and seasonal periodicity of *C. fistula*, *C. occidentalis*, and *C. tora* pollen grains in the atmosphere of West Bengal, India (in Sriniketan, a suburb of Calcutta) for two consecutive years (July 2004–June 2006), (2) to observe the prevalence of sensitization to these pollen grains among the respiratory allergic patients by in vivo and in vitro assays, and (3) to study the protein profile of the three pollen extracts by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) for the first time. It will be useful in the identification of the various immunoglobulin E (IgE) binding proteins present in these pollen grains and hence in allergen immunotherapy.

Materials and methods

Aerobiological sampling

An aerobiological survey for two consecutive years (July 2004–June 2006) was carried out at

Sriniketan (23.66°N and 87.66°E, 49 m above the ground level), a town about 150 km northwest of Calcutta, in the state of West Bengal, in eastern India (Fig. 1). The flora around the site is typical of that of tropical moist vegetation, and it represents the vegetation associated with the Indo-Gangetic plains. A Burkard personal 1-day volumetric sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK) was placed at a height of 4 m above the ground level in the sampling area to monitor the air for the entire sampling period. It was run for 10 min twice in a day between 0700 to 0800 hours and 1700–1800 hours. The exposed slides were scanned high-resolution light microscope (Leitz, Diaplan, Germany) at $\times 400$ as per the guideline of The British Aerobiology Federation (1995) to study the seasonal variation of relevant pollen grains.

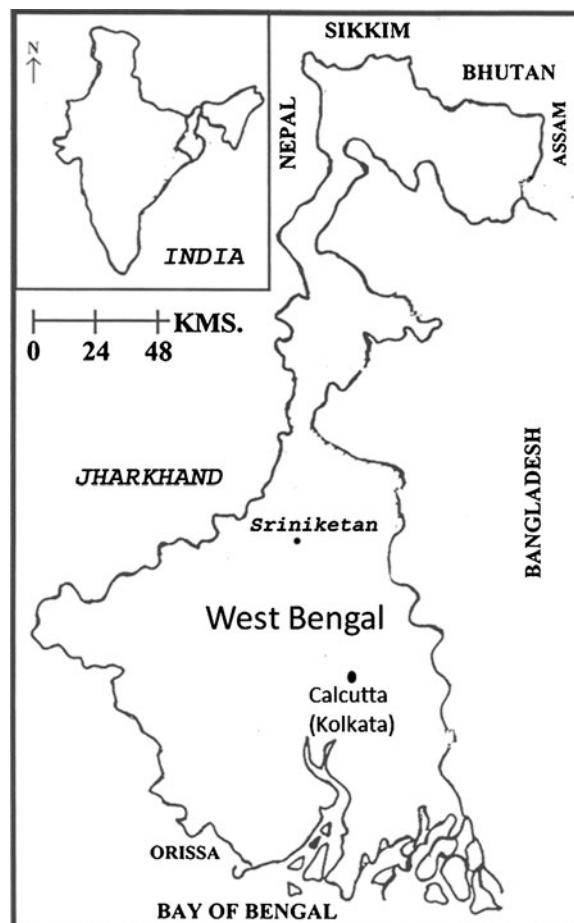


Fig. 1 Geographical location of Calcutta city, West Bengal, India, and the sampling site (Sriniketan)

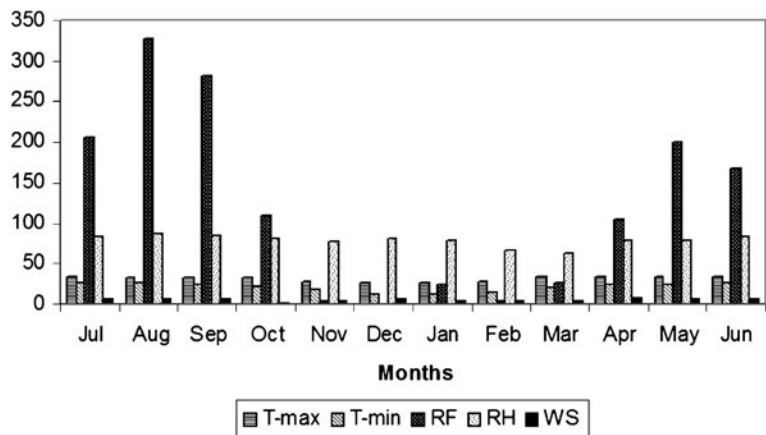
The daily pollen concentration was expressed as number of pollen grains per cubic meter of air. The identification of airborne pollen grains was done by comparing the pollen types with the reference palynological collection (of the Palynology Section, Department of Botany, Bose Institute, Calcutta, India) prepared from the local flora and by specific bibliographic consultations (Erdtman 1952; Erdtman 1969; Faegri and Iversen, 1964; Huang 1972).

For scanning electron microscopic (SEM) studies, pollen grains suspended in a drop of water were directly transferred with a fine pipette to a metallic stub using double-sided cello tape and coated with gold in a sputtering chamber. Coating was restricted to 150 Å. The SEM examination was carried out on a Quanta™ Scanning Electron Microscope (FEI, Quanta 200, the Netherlands). The measurements are based on 10 readings from each specimen.

Climate and meteorological parameters

Meteorological data of the study area were collected from the Meteorological station, Sriniketan, which is about 500 m far from the sampling site. Sriniketan has a sub-tropical monsoon climate, with five distinct seasons: (a) monsoon (July to September), (b) autumn (October), (c) winter (November to January), (d) spring (February to March), and (e) summer (April to June). The following data were recorded daily for the 24-month period of the study: maximum and minimum temperature expressed as degree Celsius, rainfall in millimeters, wind speed in kilometers per hour, and relative humidity expressed as percent saturation (Fig. 2).

Fig. 2 Meteorological parameters of Sriniketan (July 2004–June 2006): temperature (maximum, $T_{\max}$, and minimum, $T_{\min}$) expressed in degree Celsius, rainfall (RF) in millimeters, relative humidity (RH) in percent, and wind speed (WS) in kilometers per hour



Statistical analysis

The relationship between the meteorological parameters (temperature, relative humidity, average wind speed, and average rainfall) and monthly pollen concentration was calculated using Spearman nonparametric correlation coefficients. The Statistical Package for Social Sciences software (version 10.0) has been used for statistical analysis by conducting Student *t* test. The $p < 0.05$ and $p < 0.01$ were considered to be significant.

Preparation of pollen extract

The fresh pollen samples were collected from the mature anthers of the selected plant species. The batches used throughout the work contained $< 1\%$ non-pollen impurities. They were defatted with diethyl ether, and then 10 g of them was extracted in phosphate buffer saline (PBS; 0.1 M Na-phosphate, 150 mM, pH 7.2) by continuous stirring at 4 °C for 16 h in 1:10 (w/v) ratio. After centrifugation at 14,000×g, the clear supernatant was dialyzed and passed through a 0.22-μm Millipore filter (Millipore Corp., USA). The filtrate was then lyophilized and stored at −70 °C in aliquots.

Biochemical analysis

The total soluble protein content of whole pollen extract of each pollen type was determined according to Lowry et al. (1951). Total lipid was estimated using the method of Itoh and Kaneko (1974). Total soluble carbohydrate content was determined by the method of Duboise et al. (1956).

Skin prick test and sera collection

Skin tests performed a set of airborne allergenic pollen grains along with *C. tora*, *C. occidentalis*, and *C. fistula* pollen grains on polysensitized patients attending the Allergy Unit of The Institute of Child Health, Calcutta, selected because they all had demonstrated clinical manifestations of seasonal rhinoconjunctivitis and/or bronchial asthma. All these patients reported having *Cassia* tree growing in their locality. The detailed history including age, sex, family history, onset, and duration of symptoms were recorded. The allergen extracts were prepared by extracting the pollen at 95 % purity with 0.1 M PBS (1:50 w/v) (Sheldon et al. 1967). Histamine phosphate (1 mg/ml) and PBS (1 mg/ml) were used as positive and negative controls, respectively. Tests were performed with 20 µl pollen extract placed on the ventral side of the forearm with a 26-G disposable hypodermic needle. According to International guidelines, positivity was defined as mean wheal diameter ≥ 3 mm compared with negative control (Dreborg and Frew 1993). The reaction was graded from +1 to +3 level (+1=erythema, 20 mm in diameter, +2=wheal and erythema >20 mm in diameter, +3=wheal >3 mm and erythema) according to Stytiš et al. (1982). Sera were collected from the patients with +2 or +3 level skin reaction to whole pollen extract. None of these patients were receiving immunotherapy during the time of sera collection. Control sera were collected from non-atopic healthy volunteers (confirmed by negative skin reaction to the panel of allergenic pollen extracts and IgE-enzyme linked immunosorbent assay (ELISA), as described later) and having no history of allergic or systemic diseases in them. The study was approved by the Ethics Committee of the hospital, and written informed consents were obtained from the subjects before their participation.

ELISA

ELISA was performed to measure the pollen-specific IgE levels with the crude pollen extract against pooled patients' sera according to Engvall and Pearlman (1971). Antihuman IgE-alkaline phosphatase conjugate (Sigma Chemical Co., USA) was used as secondary antibody, and pNPP was used as substrate. The absorbance was measured with an ELISA reader at 405 nm. The individual patient having P/N (the ratio

of OD of patient sera with respect to control) value 3.5 was selected for further study.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

The SDS–PAGE of crude pollen extract was carried out on 12 % acrylamide gel using discontinuous buffer system according to Laemmli (1970). The extraction of the soluble protein was made in sample buffer (0.5 M Tris–HCl, pH 6.8, 2 % SDS, 5 % glycerol, 5 % β -mercaptoethanol, 0.1 % bromophenol blue dye) with heating for 5 min at 100 °C before loading. The gel run was made in Tris–glycine buffer (pH 8.3) with 0.1 % SDS. The gel was calibrated with molecular weight markers.

Results

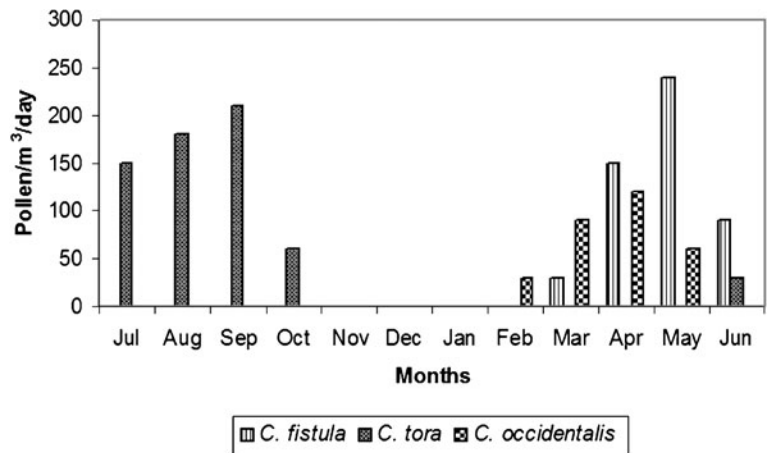
Aerobiological survey

Cassia pollen was found to be present from February to October, comprising of different species in different time round the year. Among these, *C. fistula* pollen is present in the air during the month of March to June with its peak concentration ($240 \text{ m}^{-3} \text{ day}^{-1}$) in May. The incidence of *C. tora* pollen was recorded in June to October with a peak ($210 \text{ m}^{-3} \text{ day}^{-1}$) during September, while *C. occidentalis* pollen was found from February to May with a peak ($120 \text{ m}^{-3} \text{ day}^{-1}$) during April (Fig. 3).

Microscopic studies revealed that *C. fistula* is oblate-spheroidal to sub-prolate in shape, $25\text{--}27 \times 29\text{--}35 \text{ }\mu\text{m}$ in size (Fig. 4a). *C. tora* pollen is sub-prolate to oblate spheroidal in shape, $32\text{--}42 \times 30\text{--}35 \text{ }\mu\text{m}$ in size with scabrate process (Fig. 4b). *C. occidentalis* is prolate to oblate spheroidal in shape, $32\text{--}47 \times 35\text{--}45 \text{ }\mu\text{m}$ in size (Fig. 4c).

The pollen concentration depends upon the meteorological factors, and this has been statistically supplemented. A correlation was found between the monthly pollen counts (Table 1) and meteorological factors. Using Spearman correlation test, *C. fistula* pollen was found to be positively correlated with maximum temperature at 0.01 level and with wind speed at 0.05 level. *C. tora* pollen was found to be positively correlated with minimum temperature, RH, and rain fall at 0.01 significant level, while *C. occidentalis* was

Fig. 3 Average monthly concentration of three pollen types (July 2004–June 2006)



negatively correlated with RH at 0.05 significant level (Table 1). The air concentration of each pollen type more or less coincided with their respective flowering period.

Biochemical analysis

The chemical studies of these pollen types revealed that the percentage of protein, carbohydrate and lipid is quite similar in each type with slightly high amount of protein (11.06 %) and carbohydrate (21.1 %) in *C.*

fistula. *C. occidentalis* had slightly higher concentration (17.2 %) of lipids (Table 2).

Skin prick test and ELISA

From the skin prick test with the whole pollen extract, maximum allergic sensitivity was noted in *C. tora* pollen (34.7 %) with 30.5 % at +1 level and 4.16 % at +2 level response. In *C. fistula*, 33.3 % sensitivity was noted with 30.89 % at +1 level and 2.43 % at +2 level of response. Among the three *Cassia* species, *C.*

Fig. 4 Scanning electron micrograph of **a** *C. tora*, **b** *C. fistula*, and **c** *C. occidentalis*

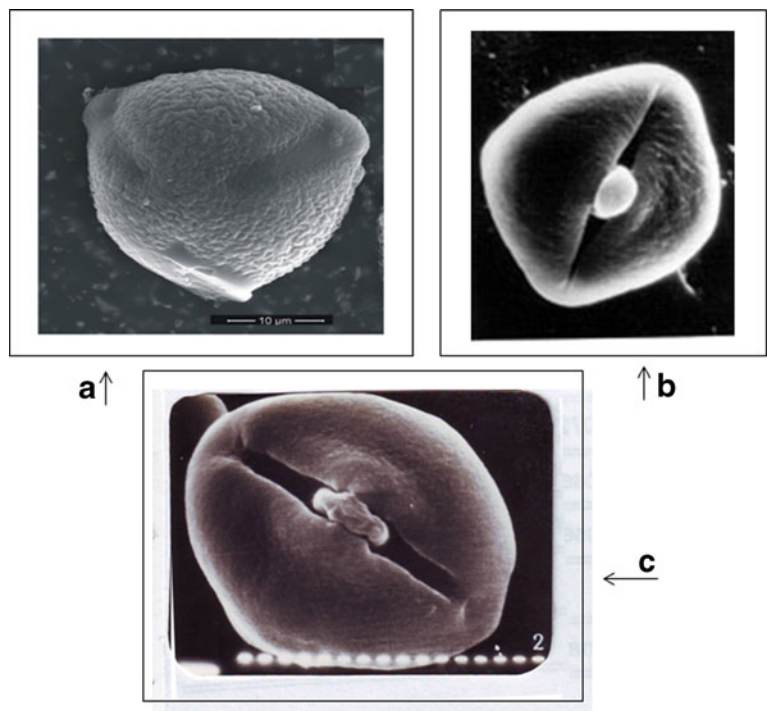


Table 1 Correlation between the pollen concentration and the main meteorological parameters by using Spearman correlation test

Meteorological parameters	<i>C. fistula</i>	<i>C. tora</i>	<i>C. occidentalis</i>
$T_{\max}$	0.795**	0.195	0.420
$T_{\min}$	0.225	0.749**	-0.112
RF	0.141	0.811**	-0.129
RH	-0.212	0.866**	-0.645*
WS	0.637*	0.023	0.337

$T_{\max}$ maximum temperature, $T_{\min}$ minimum temperature, RF rainfall, RH relative humidity, WS wind speed

* $p < 0.05$; ** $p < 0.01$ (levels of significance)

occidentalis elicited minimum 28.5 % response, of which 26.7 % at +1 and 1.78 % at +2 level (Table 3). Sera were collected for ELISA from patients showing positive reaction to specific crude pollen extract on SPT. The serum of each patient was tested individually against corresponding pollen allergen to detect the presence of specific IgE titer. The result of ELISA was expressed in terms of P/N value. Four out of ten patients showed high level of IgE titer against *C. fistula* allergen (Fig. 5a), while three patients showed high P/N value (>3.5) against seven tested sera of *C. occidentalis* positive patients (Fig. 5b). In case of *C. tora*, four out of 12 patients' sera showed high IgE titer (Fig. 5c).

SDS-PAGE

The results of 12 % SDS-PAGE of soluble protein of the three pollen extracts demonstrate a number of bands in all the cases. *C. tora* showed a total 10 number of bands of molecular weights 77.2, 66.8, 57, 48, 44, 39.2, 33.5, 22.7, 17.5, and 15.8 kDa (Fig. 6 B). *C. fistula* showed 11 protein bands of

molecular weight 81.8, 73.9, 66.8, 60.4, 53.0, 46.6, 44.0, 38.7, 34.5, 28.6, and 22.7 kDa (Fig. 6 C), while *C. occidentalis* showed 11 major protein bands of molecular weight 66.8, 63.0, 55.4, 45.3, 51.6, 45.3, 42.1, 32.5, 30.2, 23.7, 21.1, and 16.3 kDa (Fig. 6 D).

Discussion

Pollen grains are an established cause of respiratory allergy (Main 2003; Bousquet et al. 2010a). *Cassia* pollen grains are dominant in the atmosphere during their flowering season (February to October) and have been reported from different zones of India (Agashe and Anand 1982; Ahlawat et al. 2010; Anonymous 2000; Chauhan and Goyal 2006; Mandal et al. 2008; Sahney and Chaurasia 2008; Satheesh et al. 1994; Sharma et al 2010; Tripathi et al. 2004).

Until now, *C. siamea* has been the most studied species of the genus *Cassia* (Parui et al, 2002). In the present study, an attempt has been made to detect the allergenicity of the airborne pollen grains of three species of *Cassia* (*C. fistula*, *C. occidentalis*, and *C. tora*) which grow wildy in the tropical and subtropical parts of India. They were trapped by Burkard personal 1-day volumetric sampler, and their concentrations in air were expressed as pollen per cubic meter per day as a monthly average for each case. During the periods of maximum concentration, *C. fistula* pollen contributed a high amount ($240 \text{ m}^{-3} \text{ day}^{-1}$) in the month of May with the frequency between March and June. The incidence of *C. tora* pollen was recorded in June to October with a high concentration ($210 \text{ m}^{-3} \text{ day}^{-1}$) in September, while *C. occidentalis* pollen was found from February to May with peak concentration ($120 \text{ m}^{-3} \text{ day}^{-1}$) in April. This study is based on data from two consecutive years only and is open to refinement with more data, which will be obtained from more years of sampling. This could help in the establishment of flowering forecasts of various species of *Cassia* and help clinicians in the diagnosis and therapy of respiratory allergic patients.

Meteorological parameters such as temperature (minimum and maximum), rainfall, RH, and wind speed have profound effect on airborne pollen concentration (Andersen 1980; Bricchi et al. 1992; Rodriguez-Rajo et al. 2003). Thus, the composition of airspora in a particular area changes qualitatively and quantitatively with seasons (Hirst 1953). In the

Table 2 Biochemical analysis of the three pollen types (percentage of dry weight)

Pollen types	Total protein (%)	Total lipid (%)	Total carbohydrate (%)
1 <i>Cassia fistula</i>	11.06	14.0	21.1
2 <i>Cassia occidentalis</i>	9.46	17.2	18.7
3 <i>Cassia tora</i>	9.00	15.0	19.5

Table 3 Clinical characteristics of the patients selected for the study

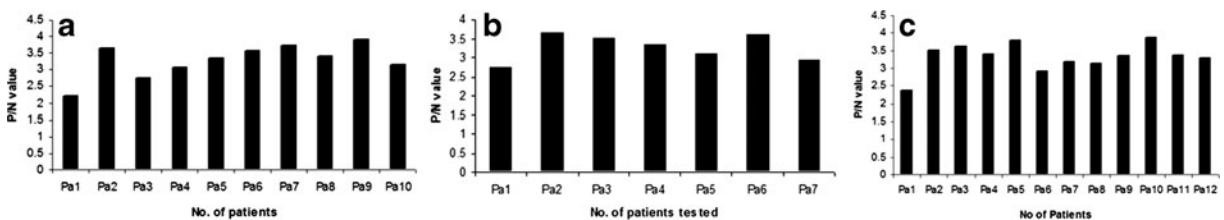
Sl. no.	Pollen type	Total no. of tested patient	No. of positive patient (% response)	Intensity of reaction	
				+1	+2 or more
1	<i>Cassia fistula</i>	123	41 (33.3)	38 (30.89)	3 (2.43)
2	<i>Cassia occidentalis</i>	56	16 (28.5)	15 (26.7)	1 (1.78)
3	<i>Cassia tora</i>	72	25 (34.7)	22 (30.5)	3 (4.16)

present paper, a correlation was made between the monthly pollen counts and meteorological factors using Spearman correlation test. From the values of correlation coefficient (r), it was found that *C. tora* pollen was positively correlated with minimum temperature, rainfall, and RH at 0.01 level of significance. The pollen concentration of *C. fistula* was positively correlated with maximum temperature and wind speed. The level of significance for maximum temperature was 0.01, while it was 0.05 for wind speed. *C. occidentalis* pollen was negatively correlated with RH at 0.05 level of significance. Similar results have also been observed in India (Agashe and Alfadil, 1989; Banik and Chanda 1990; Boral et al. 1999; Singh and Singh 1994). The meteorological parameters could be useful in the preparation of predictive models of the pollen concentration of *Cassia* pollen grains. Spearman's coefficient explains that temperature is the meteorological condition that best explains atmospheric pollen concentration variations, showing the fundamental importance of its effect on the dispersion and transport of pollen grains (Stefanic et al. 2005). These factors have a substantial effect on the variation of *Cassia* pollen grains in the atmosphere. The meteorological parameters could be useful in the preparation of predictive models of the pollen concentration of *Cassia* sp. Information on the onset of the allergenic pollen season in an area might be used to warn patients suffering from respiratory allergy.

The chemical analysis revealed the highest percentage of protein and carbohydrate in *C. fistula* and the highest amount of lipid in *C. occidentalis*. Similar results have been observed in case of *C. siamea* and *C. alata* in previous studies (Mondal et al. 1998; Sharma et al. 2009).

The results of SPT and ELISA demonstrate the allergic potential of pollen of three species of *Cassia* on polysensitized patients attending the allergy clinic. Polysensitization is more prevalent than monosensitization in the general population as suggested in various surveys (Bousquet et al. 2010b; Calderón et al. 2012). The maximum allergic sensitivity was noted in the pollen of *C. tora* (34.7 % positivity), followed by *C. fistula* (33.3 % positivity). Pollen grains of *C. alata* and *C. siamea* were clinically reported to be allergenic by other studies, showing about 46 to 55 % positivity in skin reaction tests (Boral et al 2004; Parui et al 2002; Sharma et al 2009).

The results of 12 % SDS-PAGE analysis for the soluble protein (Fig. 6) exhibited a number of bands in all three pollen types. A total 10 major protein bands were found in *C. tora*, and 11 major protein bands were observed both in *C. fistula* and *C. occidentalis* with a molecular range between 15.8 and 81.8 kDa. Similar results were shown from studies on other species of *Cassia* (*C. alata* and *C. siamea*), which resolved in to 11 to 23 protein bands in the range of 17–100 kDa (Parui et al 2002; Sharma et al 2009).


Fig. 5 Results of ELISA in terms of P/N value for **a** *C. fistula*, **b** for *C. occidentalis*, **c** for *C. tora* (Pa: Patients)

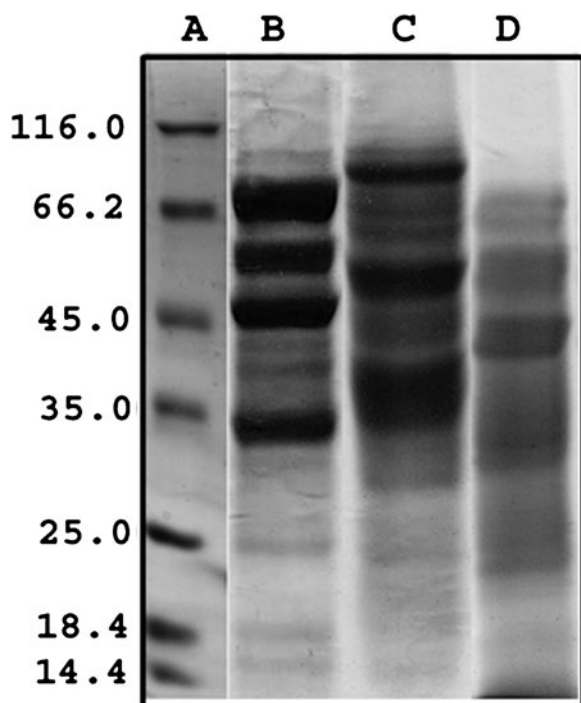


Fig. 6 Twelve percent SDS–PAGE of total soluble protein of three pollen types: *A* molecular weight marker, *B* crude pollen extract of *C. tora*, *C* crude pollen extract of *C. fistula*, and *D* crude pollen extract of *C. occidentalis*

These findings with the three species of *Cassia* pollen allergens open the scope of further work to determine the specific IgE binding protein or any cross reactive protein present between the phylogenetically related species. Allergen characterizations/cross-reactivity studies are also reported from other members of Fabaceae such as *Acacia* (Howlett et al 1982), *C. occidentalis* (Satheesh et al. 1994), *C. siamea* (Parui et al. 2002), *D. regia* (Mandal et al. 2009), *P. pterocarpum* (Mandal et al. 2011), and *Prosopis julifera* (Thakur et al. 1991) pollen grain. IgE cross-reactivity is of strong clinical interest, since it is the common cause of IgE-mediated polysensitization in susceptible population (Valenta et al, 1996). Various protein/glycoprotein components present in taxonomically close or distant plant species could be responsible for this cross-reactivity.

In conclusion, it can be said that the pollen of *Cassia* spp. (*C. fistula*, *C. occidentalis*, *C. tora*) are significant contributors of aeroallergen load of the study area in West Bengal. The protein profiles of these three pollen have been studied for the first time. Detailed studies regarding the identification and purification of their

IgE-binding components will be useful in allergen immunotherapy of susceptible subjects.

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