



## Identification of Some Allergenic Pollen Proteins of Some Fabaceae and Poaceae Species using SDS-PAGE Technique

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### ABSTRACT

The current study aims to identify the allergenic pollen proteins of some plant species of Fabaceae and Poaceae families by SDS-PAGE technique to know which of them can cause allergy. Six different plants belonging to Fabaceae family (*Bauhinia variegata*, *Cassia javanica*, *Delonix regia*, *Peltophorum africanum*, *Senna didymobotrya* and *Senna surattensis*) and four different plants belonging to Poaceae family (*Avena sativa*, *Setaria viridis*, *Sorghum bicolor* and *Zea mays*) were collected from two different zones. The 1<sup>st</sup> site is located at Cairo Governorate and the 2<sup>nd</sup> is Kafr Elsheikh Governorate. Sampling was carried out at both two mentioned fixed sites for three consecutive periods from July 2018 to June 2021. The obtained results showed that the prolate-spheroidal was the most common pollen shape, trizonocolpoate and monoporate shapes were the most prevalent aperture and the regulate-perforate and granulate types were the most noticed sculpture. SDS showed notable bands, ranged from 25, to 75 kDa which were related to hydrophilic proteins antigen in pollens. Interestingly, SDS-Page of the serum of the patient revealed similar bands within 74, 51 and 40 kDa. Therefore, our study highlighted the possible role of pollen grains related to Fabaceae and Poaceae families in allergic patient in Egypt.

### 1. Introduction

Pollen grains, fungus spores, house dust, house dust mites, animal allergens, insect allergens, industrial allergens, food allergens, and medication allergens are the most prevalent airborne allergens <sup>[1]</sup>.

Pollen allergy, or pollinosis, is a disorder associated to the presence, in the course of the seasons, of a greater or fewer number of pollen grains from quite different plants, because of the impacts of climate change on plant allergenic species <sup>[2]</sup>.

In addition to symptoms like rhinitis and conjunctivitis, pollinosis can also result in respiratory issues of varied severity (such as an irritable cough, tracheitis, asthma, etc.) and, less frequently, skin manifestations (urticaria, eczema) [3]. The pollen grain is an essential component of the flowering plant life cycle and is responsible for fertilizing the female gametophyte [4]. It is the means by which the male gametophyte is transferred to the female reproductive organs in seed plants across all angiosperm and gymnosperm species [5]. While pollinating, the pollen grains are transported by air to the stigma [6].

Once mature grains are placed on the stigma or on artificial medium (or mucosal membranes), they swell due to water absorption (an almost passive mechanism) [7]. About 10–18% of all blooming plants are anemophilous plants, which are thought to be among the most significant pollen allergens [8]. The impact of pollen on human health is primarily evident in allergic diseases [9]. Plant pollen is considered as the main aeroallergen causing allergic reactions [10]. Pollen allergens are water-soluble proteins or glycoproteins that can quickly cause an allergic reaction mediated by the IgE antibody [11]. However, there are about of 7868 proteins found in all plants, only 29 are allergens [12]. Allergenic proteins are usually located within the pollen protoplast and readily released during the rehydration process [8]. An estimated 40% of allergy patients have experienced a pollen reaction [13].

Despite the undeniable advantages of green spaces widely cultivated for public health, some problems have arisen from urban planting due to sensitization of pollen, especially in urban settings [14-15]. Where plants use anemophilous pollination, a reproductive strategy that allows pollen grains to reach the gynoecium in female flowers for fertilisation. Although this tactic is incredibly successful for plants, it frequently poses a health danger to people [16]. About 10–18% of all blooming plants are anemophilous plants, which are thought to be among the most significant pollen allergens [17]. The hydrophilic protein or glycoprotein (antigen) in pollen, which can elicit stronger or lesser allergy reactions in people, has a molecular mass of 10 - 70 kDa and is resistant to pH changes and high temperatures [18].

Pollen grains' allergenic makeup varies depending on the pollen condition; pollen from immature flowers contains fewer allergic proteins than pollen from mature flowers, and pollen grains from hydrated and activated blooms have more allergenic proteins than pollen from mature flowers [19]. Patient features and environmental factors are the two broad types of allergy risk factors. The genetic make-up, age, sex, and race of the patient are the most important factors [20].

The most significant sources of environmental variables include exposure to infectious diseases during infancy and early childhood and environmental contaminants [20]. Nevertheless, pollens are a significant factor in the development of allergies [21]. An estimated 40% of allergy patients have experienced a pollen reaction [22]. The aim of the present study is to investigate the morphological features of the most common pollen grains related to Fabaceae and Poaceae families, electrophoresis of their proteins and correlate the obtained proteins with serum data from allergic patients in Egypt.

## 2. Materials and methods

### Materials

Immunoproteomic study was done on several ornamental plants and grasses to identify the responsible allergens Table 1.

### Selection of sampling sites

In order to explore the impact of floral composition of a specific area on its aeropalynological spectrum, the selection of sampling sites was done with the goal of correlating aerial pollen variety with the density of surrounding vegetation. Site 1: it is in Cairo and Kafr El sheikh Governorates.

### Sampling technique

From July 2018 to June 2021, sampling was done over three consecutive months at the two indicated fixed sites. At both locations, sampling took place once a week for 20 minutes above ground. Three separate periods throughout the day were used to collect samples. Before and after each usage, samplers were washed and sterilized with 70% ethanol or 90% isopropyl alcohol. After separation of extraneous materials, the anthers were dried at 27 °C, gently crushed and the pollen thus released was passed through different grades of sieves to obtain pure pollen.

**Table 1.** The taxonomic characteristic features of the examined spp including family, life form and flowering season.

Plant name	Family	Life form	Flowering season
<i>Bauhinia variegata</i>	Fabaceae	Tree	September - November
<i>Cassia javanica</i>	Fabaceae	Shrub	April - June
<i>Delonix regia</i>	Fabaceae	Tree	May- June
<i>Peltophorum africanum</i>	Fabaceae	Tree	November - February
<i>Senna didymobotrya</i>	Fabaceae	Shrub	Late winter - early spring
<i>Senna surattensis</i>	Fabaceae	Tree	September - January
<i>Avena sativa</i>	Poaceae	Grass	June - July
<i>Setariaviridis</i>	Poaceae	Grass	August – October
<i>Sorghum bicolor</i>	Poaceae	Grass	50- 150 days after planting
<i>Zea mays</i>	Poaceae	Grass	July - October

Pollen samples were then stored at -20 °C. The specimens studied were identified by means of comparison with specimens kept in the Herbarium of the Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

#### Pollen identification

Pollen identification was done by both light and scanning electron microscopes.

#### Pollen grain identification by light microscope

One of the most popular methods for mounting pollen is the glycerol jelly method. It calls for the use of glycerol jelly, which is made of 10 g of gelatin, 35 ml of distilled water, and 30 ml glycerol. Freshly collected pollens are used.

#### Pollen morphology analysis by scanning electron microscope

One of the most often utilized techniques for pollen preparation for electron microscopy is acetolysis. Nonetheless, it has been demonstrated to lead to distortions, which had an impact on the creation of a new and improved approach. Unacetolyzed pollen grains transferred to stubs, coated with gold film in the JEOL FC 1100 impregnator, and analyzed with the FEI QUANTA 400 FEG ESEM/EDAX PEASUS X4M scanning electron microscope from CEMUP are some of the best and most recent techniques.

#### Protein extraction of pollen grains

Dried pollen was suspended in phosphate buffered saline at pH 7.4 (w/v) and 4 °C in a 1:20 ratio. Through 4 hours of continuous stirring in the same buffer, soluble proteins were extracted. The suspension was then centrifuged for 30 minutes at 4 °C at 13 200 rpm. The supernatant was then centrifuged once more after passing through a 0.45 µm millipore filter.

Using the Coomassie Protein Assay Reagent (Pierce), the Bradford method was used to colorimetrically measure the amount of soluble protein in each pollen extract [23-26].

#### Quantitation of protein

The Lowry technique was used to determine the antigenic extracts' protein concentration [27]. Phosphotungstic acid was used to precipitate soluble protein in this technique. A 2 ml solution of 2% sodium hydroxide was used to dissolve the precipitate. Estimating the protein content of an aliquot was done. BSA was diluted in various ratios (0 µg/ml to 100 µg/ml) and measured against absorbance to create a standard curve. The protein content of the antigenic extracts of several pollen kinds was determined using this method.

### Determination of pollen extracts' protein profile using SDS-PAGE

Using Bio-Mini-Protean Rad's II system, the various pollen extract samples were electrophoretically separated in polyacrylamide (PAGE) gels under denatured conditions, with 0.2% SDS present [28]. Samples were denatured for 10 minutes at 80°C with denaturing solution concentrated 2x (100mM Tris/Bicine, 4% SDS (m/v), urea 6 M, 4% -mercaptoethanol (v/v), and Brilliant Blue before being applied to the gels.

A protein pre-stained standard (Precision Protein Standards from Bio-Rad) with known molecular masses between 10 and 250 kDa was applied to each gel in addition to the samples. In 10% and 12% polyacrylamide gels with an electrophoresis buffer of 100mM Tris/Bicine, 0.1% SDS, protein separation was achieved.

### Patient sera

Seven patients were chosen at random who had previously been identified as polysensitive to pollen protein extracts as determined by skin-prick tests. Their sera showed specific IgE level that ranged from very low (2.09 - 30.40 Ku/L) to very high. The study contained one adverse control. According to the manufacturer's instructions, serum was extracted from whole blood and allergen-specific serum IgE was quantified using a standard ImmunoCAP™ assay (ImmunoCAP™ specific IgE, Phadia AB).

## 3. Result

### Pollen morphology

Examined pollen grains show considerable variations in their characteristics. The polar length ranged from 4.616 to 87.388 µm, while the equatorial width ranged from 5.675 to 58.513 µm. All pollen grains have large size, except *Setaria viridis* which as small size. Regarding the pollen shape, the shape class of most pollen grains is prolate-spheroidal (4 taxa= 40. Regarding the aperture, trizonocolpoate and monoporate shapes are the most represented (4 taxa = 40%), the number of apertures in the majority of pollen grains is three (5 taxa = 50%), operculum and margo are absent in most of the studied taxa. Seven types of exine sculpture were described, regulate-perforate and granulate types were the most represented (2 taxa = 20%). The annulus is absent in most of the studied taxa (6 taxa = 60%).

The pollen morphological characters were summarized in Table 2 and some of the specific structures (micro-photographs) were arranged and illustrated in Fig. 1&2.

### Protein Profile of Pollen Extracts

The protein profile of the extracts showed bands between 75 and 10 kDa in all two sites extracts, there are marked notable bands zone, bands around 10 kDa, and 75 kDa. As shown in the Fig. 3, 4 and 5.

### Protein Content

The amount of staining has been used to assess the protein amounts. It was substantial in *Avena sativa* in site 1 and *Cassia javanica* in site II, while it low in *Bauhinia variegata* in both sites. Table 3.

### Protein extracts against allergic patients' serum

It was performed with the sera of patients that showing markedly positive allergic reactions to any of the total antigenic extracts of all pollen antigen samples. Seven allergic individuals who were polysensitized to wx1 and whose sera were tested against pollen protein extracts revealed immunolabelling of multiple IgE binding bands with varying densitometric values. In both site I and site II pollen extracts, the sera of all patients showed reactivity to two bands with molecular weights of 74 and 51 kDa. In addition, five of the patient sera examined showed the presence of a band with a molecular weight of roughly 40 kDa (B, C, D, F, and G). We found reactive bands in the serum of two patients below 15 kDa (D and E). The serum of three patients showed reactivity to a protein band with a molecular weight of about 27 kDa Fig. 6.

## 4. Discussion

The pollen grain is a component of the life cycle of flowering plants that, biologically, serves to fertilize the female gametophyte [28]. It is the means by which the male gametophyte is transferred to the female reproductive organs in seed plants across all angiosperm and gymnosperm species [29]. All the nutrients needed for plant growth and development are present in plenty in it [30,31]. Pollination, which is described as the transfer of the pollen grains to the female reproductive organ of plants, may occur by wind or insects [32]. The effects of pollen on human health are mostly noticeable in allergic conditions [33].

**Table2.** Micro-morphological characteristics of pollen grains of the studied taxa.

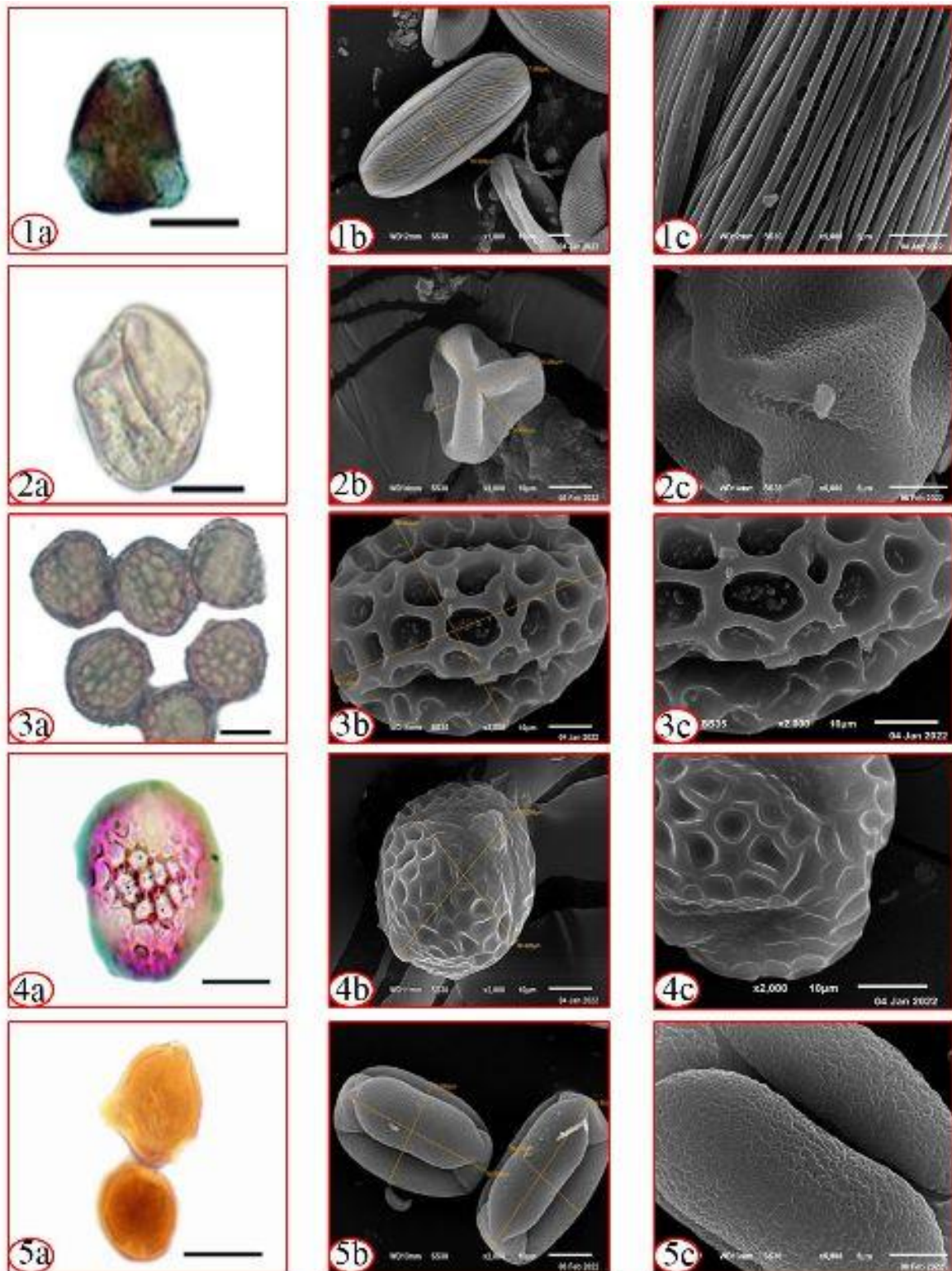
Attributes Taxa	Dimension (µm)		Pollen size (P+E / 2)	Pollen shape		Aperture			Exine sculpture	Annulus	
	Polar length (P)	Equatorial width (E)		Amb (LM)	Shape class (P/E X 100)	Shape	Number	Operculum			Margo
<i>Bauhinia variegata</i>	87.388	35.638	Large	Triangular	Perprolate	Trizonocolpate	3	-	-	Striate	-
<i>Cassia javanica</i>	26.080	24.640	large	Triangular	Prolate-spheroidal	Trizonocolporate	3	-	-	Perforate	-
<i>Delonix regia</i>	66.128	50.662	Large	Circular	Subprolate	Trizonocolpate	1	-	-	Reticulate granulate	-
<i>Peltophorum africanum</i>	39.386	36.428	Large	Circular	Prolate-spheroidal	Trizonocolporate	3	-	-	Reticulate	-
<i>Senna didymobotrya</i>	36.928	21.694	Large	Semi-circular	Prolate	Trizonocolpate	3	-	-	Regulate perforate	-
<i>Senna surattensis</i>	37.460	24.924	Large	Circular	Prolate	Trizonocolpate	3	-	-	Regulate perforate	-
<i>Avena sativa</i>	43.081	38.354	Large	Circular	Prolate-spheroidal	Monoporate	1	+	-	Granulate	+
<i>Setaria viridis</i>	4.616	5.675	Small	Circular	Subolbate	Monoporate	1	+	-	Psilate	+
<i>Sorghum bicolor</i>	45.311	42.387	Large	Semi-circular	Prolate-spheroidal	Monoporate	1	+	-	Granulate	+
<i>Zea mays</i>	69.330	58.513	Large	Semi-circular	Subprolate	Monoporate	1	+	+	Granulate	+

The majority of allergens worldwide come from pollen grains, which account for roughly 30% of the global allergenic burden [34]. The primary aeroallergen thought to be responsible for allergy responses is plant pollen [35]. Water-soluble proteins or glycoproteins, such as those found in pollen, can quickly cause an allergic reaction mediated by the IgE antibody [36]. There are only 29 protein families that are present in all plants have pollen allergens [37]. Many pollen allergenic proteins are assumed to have crucial physiological roles in pollen, particularly the pollination process, from a biological perspective [38].

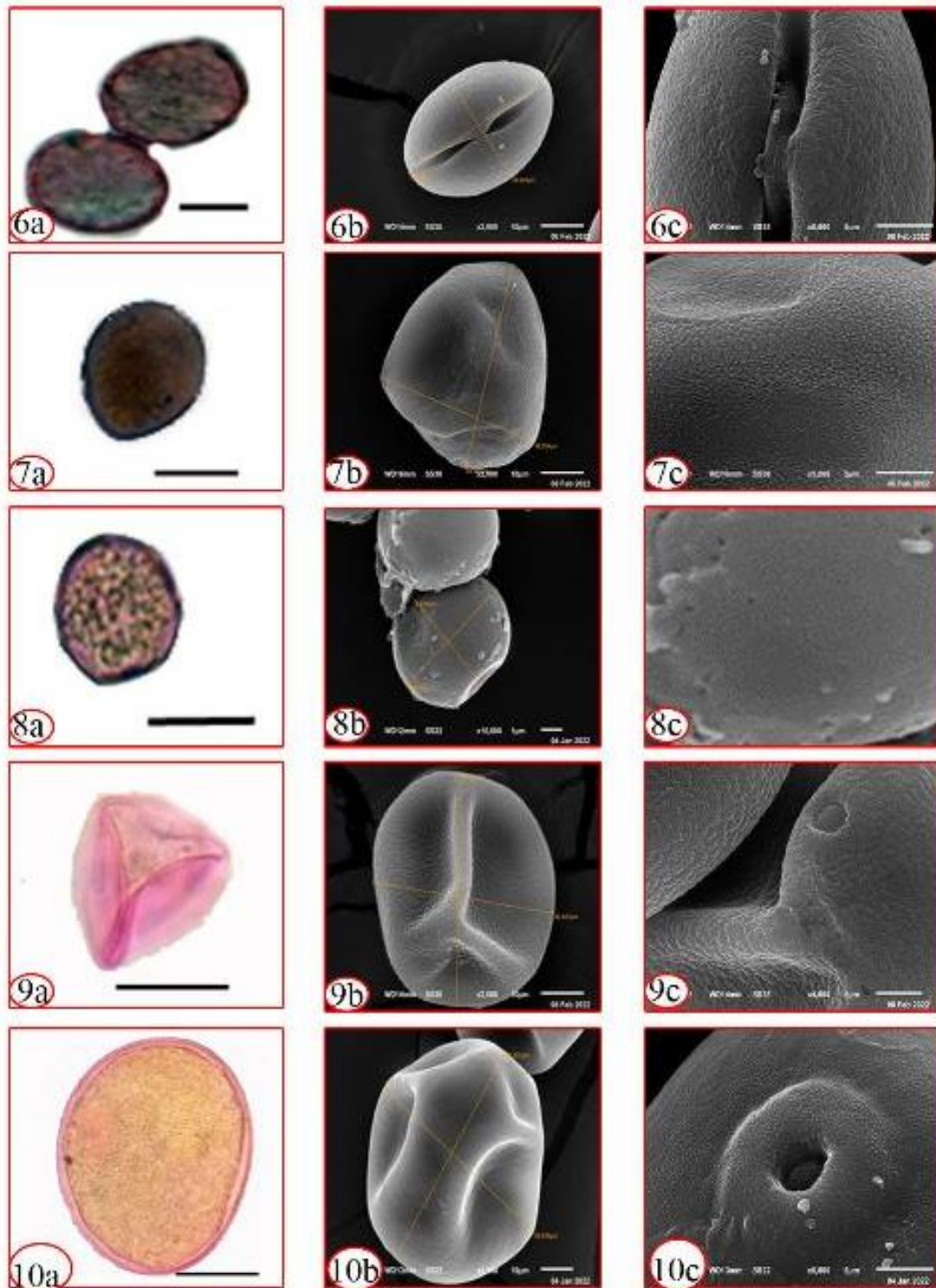
Allergenic proteins are usually located within the pollen protoplast and readily released during the rehydration process [39]. Additionally, nicotinamide adenine dinucleotide phosphate (reduced) oxidases and bioactive lipid mediators are present in pollen, and they are likely responsible for the inflammatory response [40].

Allergic reaction can also be triggered by some substances excreted by plants, such as juice and volatile oils, or in other bioaerosols of plant origin, e.g. fluids released during treatment of some plants (crop, cotton, herbs) [41,42]. The most common occurrence of type 1 allergy (immediate, anaphylactic), pollinosis, occurs when an allergen causes the body to create particular IgE-class antibodies [44, 45].

Due to the effects of climate change on plant allergenic species, pollen allergy, or pollinosis, is a disorder associated to the presence, throughout the course of the seasons, of a greater or fewer quantity of pollen grains from quite different plants [46, 47]. In addition to symptoms like rhinitis and conjunctivitis, pollinosis can also result in respiratory issues of varied severity, such as irritable bowel syndrome, tracheitis, asthma, etc., as well as less frequently, skin manifestations like urticaria and eczema [48, 49].



**Fig. 1 (1-5).** LM (a letters) and SEM (b and c letters) micro-photographs of pollen grains of the studied taxa; *Bauhinia variegata* (1), *Cassia javanica* (2), *Delonix regia* (3), *Peltophorum africanum* (4) and *Senna didymobotrya* (5).



**Fig. 2 (6-10).** LM (a letters) and SEM (b and c letters) micro-photographs of pollen grains of the studied taxa: *Senna surattensis* (6), *Avena sativa* (7), *Setaria viridis* (8), *Sorghum bicolor* (9) and *Zea mays* (10).

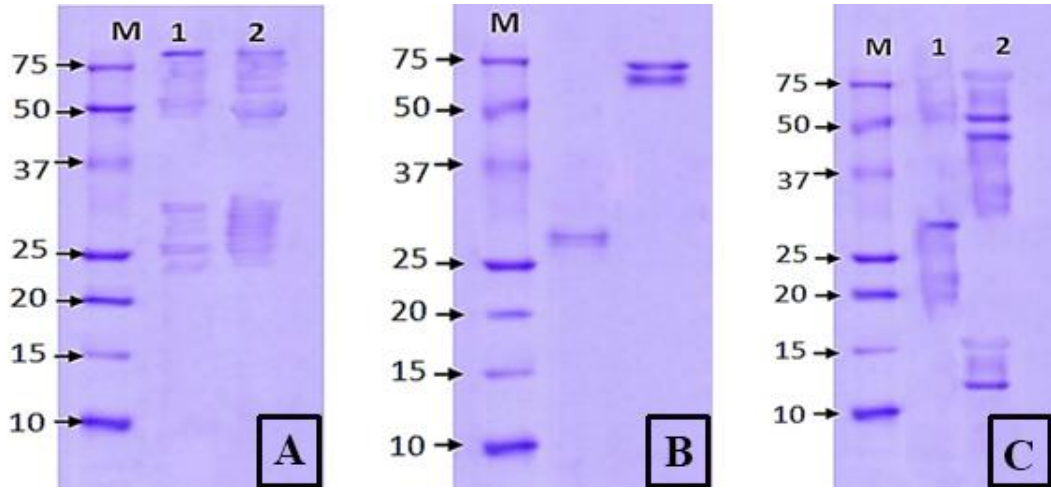


Fig. 3 Protein profile of the extracts of *Bauhinia variegata* (A), *Peltophorum africanum* (B) and *Cassia javanica* (C).

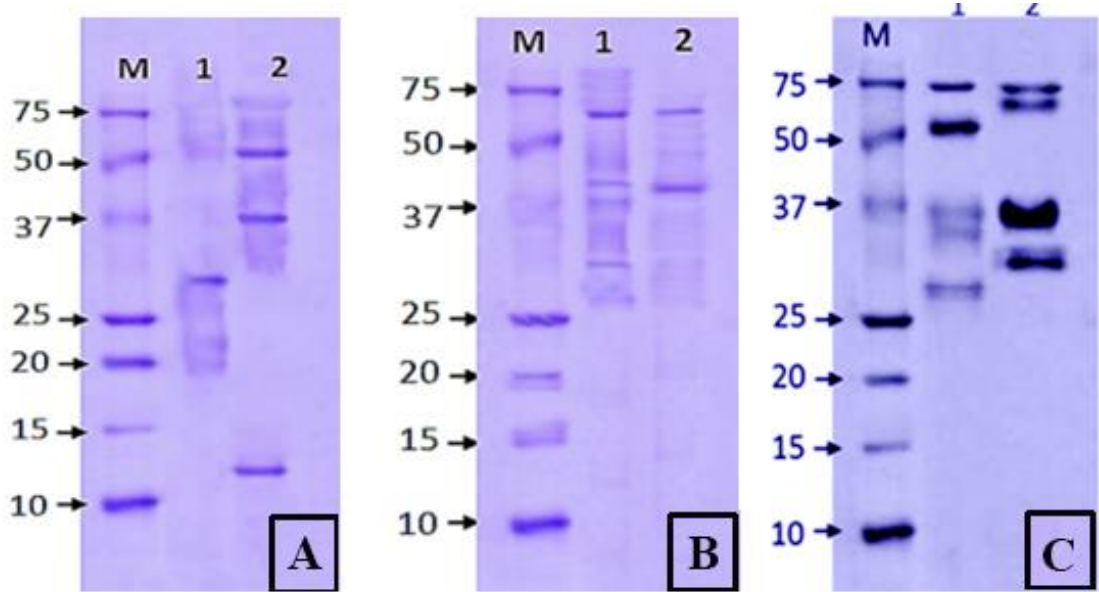


Fig. 4 Protein profiles of the extracts of *Senna didymobotrya*(A), *Senna surattensis* (B) and *Delonix regia*(C).

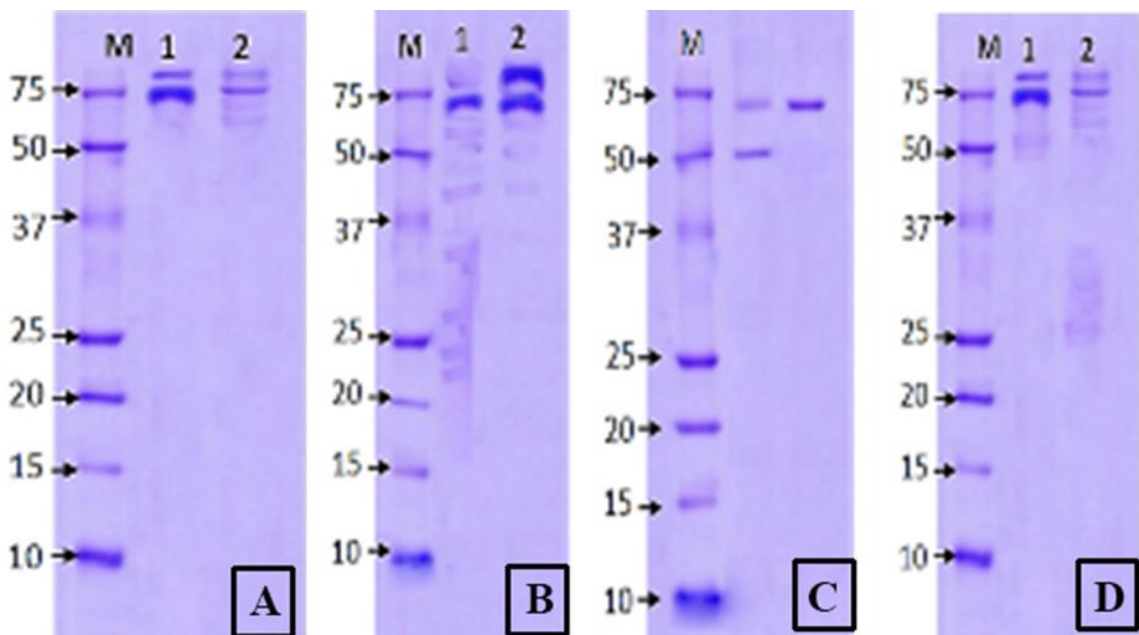
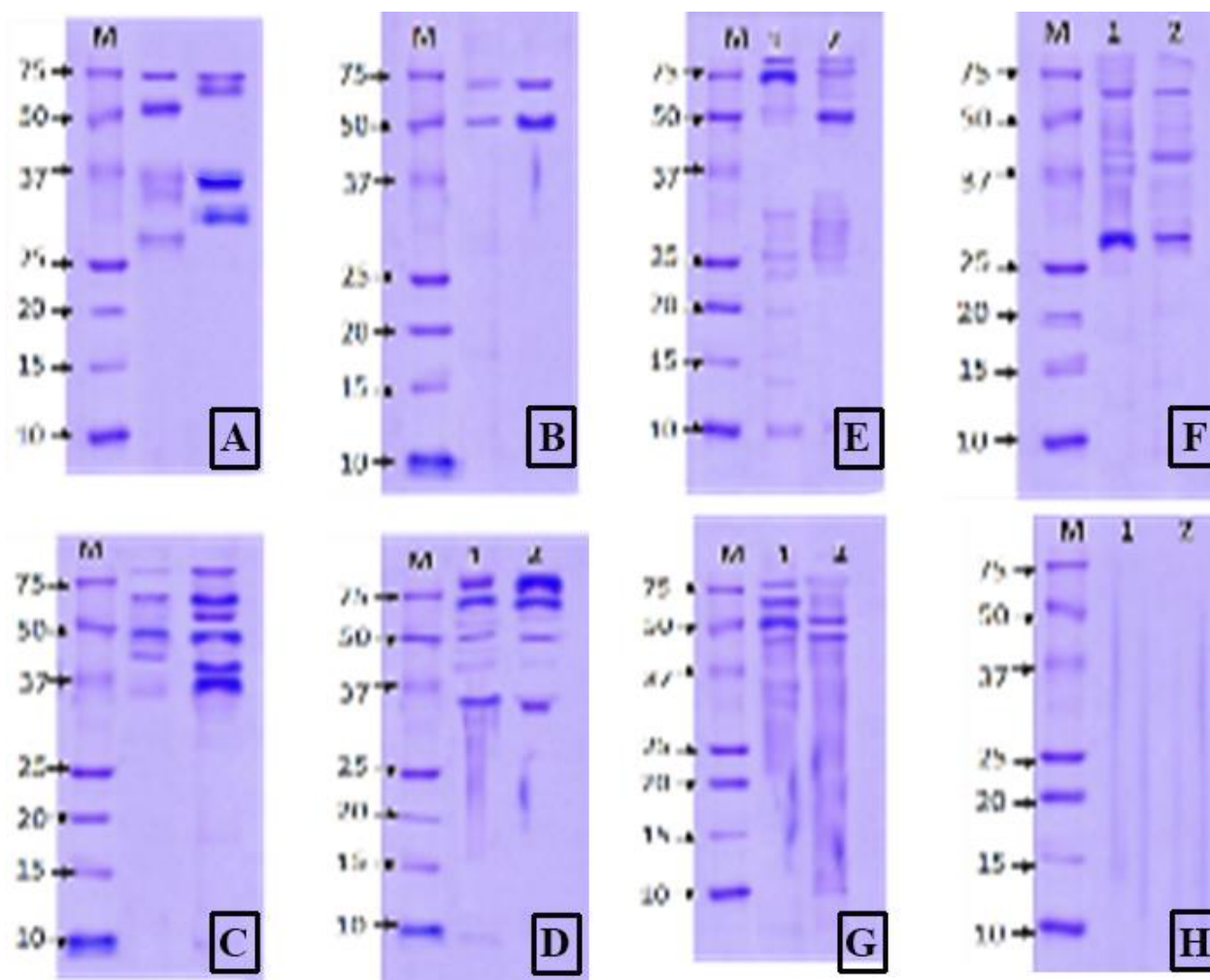


Fig. 5 Protein profiles of the extracts of *Avena sativa*(A), *Setaria viridis*(B), *Sorghum bicolor* (C) and *Zea mays*(D).



**Table 3.** Protein concentration within the different examined Taxa.

	Pollen antigen sample name	form	Protein concentration	
			Site I	Site II
1	<i>Bauhinia variegata</i>	Tree	0.53	0.75
2	<i>Cassia javanica</i>	Shrub	3.35	3.64
3	<i>Delonix regia</i>	Tree	2.27	2.19
4	<i>Peltophorum africanum</i>	Tree	0.57	0.72
5	<i>Senna didymobotrya</i>	Shrub	1.81	2.12
6	<i>Senna surattensis</i>	Tree	1.72	1.45
7	<i>Avena sativa</i>	Grass	3.85	3.55
8	<i>Setaria viridis</i>	Grass	0.83	1.09
9	<i>Sorghum bicolor</i>	Grass	1.24	1.72
10	<i>Zea mays</i>	Grass	0.67	0.83



**Fig. 6** Immunoblots of pollen proteins incubated with polysensitized patient sera with specific IgE values (A: 4.57; B: 2.09; C: 27.30; D: 7.30). Control serum (H). Lane (1): Site I pollen; lane (2): Site II pollen and lane (M): Molecular weight markers (kDa).

When airborne allergens enter the body, they stimulate the immune system, which then leads to the development of an allergic disease. Airborne allergens are biologically derived compounds. It is the proteins and glycoproteins that make up the airborne allergen that directly produce an allergic reaction [50]. Poaceae plants (grass pollen family) are the ones that cause the greatest allergies, according to the International Union of Immunological Societies (IUIS) allergen database [51]. Type I allergy associated with pollen grains of Asteraceae is common in USA and Europe, where ragweed and mugwort cause 14% and 50% of patients are suffering from pollinosis [52]. Patient features and environmental factors are the two broad types of allergy risk factors. The genetic make-up, age, sex, and race of the patient are the most important factors.

The most significant sources of environmental variables include exposure to infectious diseases during infancy and early childhood and environmental contaminants [53]. Nevertheless, pollens are a significant factor in the development of allergies [13]. Pollen contains nicotinamide adenine dinucleotide phosphate (reduced) oxidases and bioactive lipid mediators that probably contribute to the inflammatory response [54]. Certain plant excretions, such as juice and volatile oils, as well as other bioaerosols of plant origin, such as fluids generated during the treatment of particular plants, can also cause an allergic reaction (crop, cotton, herbs) [54]. The hydrophilic protein or glycoprotein (antigen) in pollen, which can elicit stronger or lesser allergy reactions in people, has a molecular mass of 10 kDa to 70 kDa and is resistant to pH changes and high temperatures [55].

Our current investigation demonstrated that there was a comparison between the protein profiles of the two sites' sample extracts, which exhibited pollen components. The published findings, however, are paradoxical; they did not discover any appreciable variations in pollen gathered from regions with various levels of environmental pollution, and they came to the conclusion that pollen grains from less contaminated areas have lower protein contents [56]. Although some protein bands appear to be missing from the pollen from one site in our investigation, distinct protein profiles were found in the pollen from the two sites. Previous investigations have found differences in the protein profiles of pollen from polluted and non-polluted areas, with pollen from the latter having less soluble protein [57].

The present study aims to identify allergenic proteins in the pollen of some taxa of Fabaceae and Poaceae plant families using SDS-PAGE technique. Our results demonstrated the presence of several protein bands in the pollen samples of all examined taxa. However, the molecular weight and intensity of the protein bands varied among the different taxa. The SDS-PAGE technique allowed us to identify several potential allergenic proteins based on their molecular weight, as reported in previous studies. For instance, the pollen of Fabaceae family contained several protein bands ranging from 30-70 kDa, which correspond to known allergenic proteins, such as vicilin, convicilin and legumin. These proteins have been reported to cause allergic reactions in sensitive individuals [58,59].

The differential in protein expression between rural and urban settings may be responsible for this decline. The antigenic profile used in our investigation was determined by immunoblot, which revealed a consistent pattern of bands for the seven zones. These findings diverge from those in the published research. Nonetheless, it is important to note that in the cited studies, the characterization of allergenic extracts was done using patients' sensitized antibodies [60]. The impact of environmental factors on vegetation and the presence of urban toxins from automobile traffic would be responsible for the differences in shape and protein composition that were detected.

In general, the extracted pollen proteins were compared to the sera of the seven allergic patients and revealed varying degrees of sensitivity. This could mean that different levels of sensitivity to the same pollen type exist depending on where it comes from, in our example, stronger sensitivity to the pollens from the two sites. Varying sensitization levels may be associated with pollen extracts obtained in urban environments having a stronger chemotactic activity on human neutrophils.

These results are consistent with recent literature demonstrating an increase in allergy prevalence across habitats as a result of air pollution [61]. Because to their tiny size and great abundance, pollen allergens are unfortunately impossible to avoid and may produce pollen-food and pollen-fruit disorders through cross-reactivity [62].

## 5. Conclusion

Pollen allergy has a remarkable clinical impact all over the world. The allergy symptoms result from immunological response of the sensitized individuals following exposure to pollen. In the present work, sampling of the airborne pollen was carried out for three consecutive years (July2018-June2021). This study provides details of allergenic pollen in bright field microscopy and express their detailed morphological diversity. Pollen focal series give the clear picture of the morphology of different pollen grains to establish a reference content. Field emission by scanning electron microscope has given detailed focus on sculpture and identification features have been highlighted to establish an identification key.

Identification of pollen proteins was carried out using SDS-PAGE technique. The protein profile of the extracts showed bands between 10 and 75 kDa, there were three marked notable bands, the first band around 37kDa, the second band at 50 kDa and the third at 75 kDa. Regarding the protein extracts against the serum of allergic patients, it was performed with the sera of patients that showing markedly positive allergic reactions. All Patients' sera analyzed presented reactivity to two bands with molecular weight ranged between 51 and 74 kDa.

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