Pólen de *Parietaria judaica*: Aerobiologia e alergenicidade

Parietaria judaica *pollen: Aerobiology and allergenicity*

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ABSTRACT

Introduction: *Parietaria judaica* pollen is able to induce severe allergic symptoms, mainly due to its high pollen output and the long persistence in the atmosphere. **Aim:** We intended to study the aerobiology and allergenic proteins of *Parietaria judaica* pollen collected in an urban area of the Porto region. **Methods:** We adopted aerobiological and immunological techniques to study the annual airborne pollen concentrations from 2003 to 2012 and the immunoreactivity of *P. judaica* pollen extracts against 21 sensitized patient sera. **Results:** *Parietaria* spp. pollen is present in the atmosphere of Porto all year long, representing 17% of the total pollen spectrum. The highest airborne pollen concentrations were mainly found from April-August. From the selected patients with pollen allergy, 34.2% showed sensitization to *Parietaria* spp. pollen extracts. Among these, 84.5% were only positive to *Parietaria judaica* and 8.5% to its counterpart *Parietaria officinalis*. The most prevalent protein bands of *P. judaica* pollen extracts when tested against allergic patient sera were those around 55 kDa, 36-30 kDa, 14 kDa and 12-11 kDa. An anti-Par j 1/Par j 2 immunoblot revealed two protein bands around 12 kDa and two possible glycoprotein bands of around 22 and 18 kDa were also showed in the *P. judaica* pollen extracts. **Conclusions:** *Parietaria* spp. is the most abundant pollen type in the atmosphere of Porto and its highest concentrations are found at the first sunlight hours. *P. judaica* in particular sensitizes a considerable fraction of pollen-sensitive individuals and several reactive protein bands were recognized by *P. judaica* sensitive IgE sera. This pollen appears to be an important source of weed-sensitization in this region.

Keywords: Aerobiological profile, allergens, Parietaria judaica, pollen, sensitization.

RESUMO

Introdução: *O pólen de* Parietaria judaica *é capaz de induzir doença respiratória alérgica, principalmente devido à elevada produção de pólen e persistência na atmosfera.* ***Objectivo:*** *Estudámos a aerobiologia e as proteínas potencialmente alergénicas do pólen de* P. judaica *recolhido na área urbana do Porto.* ***Métodos:*** *Adoptámos técnicas aerobiológicas e imunológicas de forma a estudar o espectro polínico entre 2003 e 2012 e o perfil reactivo de extractos proteicos de pólen de* P. judaica *quando incubados com 21 soros de indivíduos alérgicos a pólen, previamente seleccionados.* ***Resultados:*** *O pólen de* Parietaria *spp. está presente na atmosfera praticamente todo o ano, representando 17% do espectro polínico total. A concentração de pólen mais elevada foi verificada entre Abril e Agosto. Entre indivíduos alérgicos a pólen, 34,2% mostrou sensibilização a extractos polínicos de* Parietaria *spp. Destes, 84,5% mostraram apenas reacção positiva a* Parietaria judaica *e 8,5% a* Parietaria officinalis. *Após incubação com os soros seleccionados, os* immunoblots *de extracto de pólen de* P. judaica *apresentaram bandas proteicas reactivas mais prevalentes de aproximadamente 55 kDa, 36-30 kDa, 14 kDa e 12-11 kDa. A incubação de um* immunoblot *de extracto de pólen de* P. judaica *com anti-Par j 1/Par j 2 revelou a existência de duas bandas proteicas aproximadamente de 12 kDa. Foram também detectadas duas bandas proteicas glicosiladas no extrato de* P. judaica*, uma de cerca de 22 kDa e outra aproximadamente de 18 kDa.* ***Conclusões:*** *O pólen de* Parietaria *spp. é o mais abundante na atmosfera do Porto, surgindo em concentrações mais elevadas durante as primeiras horas de luz solar. O pólen de* P. judaica *sensibiliza uma fracção considerável dos doentes com alergias respiratórias e os soros de doentes sensibilizados demonstraram marcação de vários componentes de ligação a IgE. Assim, o pólen de* P. judaica *parece ser uma importante causa de sensibilização a herbáceas na região do Porto.*

Palavras-chave: *Alergénios, Parietaria judaica, perfil aerobiológico, pólen, sensibilização.*

INTRODUCTION

Pollen related respiratory allergies, or as common known pollinosis, is a general health problem worldwide, affecting life quality of Earth inhabitants. They are associated to an allergic response of patients to the pollen grains of several trees, grasses and weeds. Sensitization, which is the exposure to allergens, starts the immune response in allergy by eliciting the production of immunoglobulin E antibodies.

Pollen grains are biological structures produced by higher plants. They are transported from the anthers to the stigma of the same flower or of a different flower of the same species by various abiotic and/or biotic agents. This dependence on environmental conditions for successful fertilisation led to anemophilous plants developing compensatory mechanisms, such as the release of large amounts of airborne pollen and production of more aerodynamic pollen grains, making dispersal easier. These characteristics, associated to the existence of allergens in the pollen wall are the main cause triggering respiratory allergic reactions. To estimate the prevalence of pollen allergy in people who present such symptoms and to measure allergen exposure, clinicians carry out sensitization studies on a regular basis.

The development of aerobiological studies is important for the elaboration of pollen calendars, which are indicative of the presence and prevalence of allergenic pollen in the atmosphere of a sampling area. These studies allow, after several years of monitoring, estimating the date for the flowering season of plants. This knowledge is useful for clinicians in order to adequate

the standard skin prick test battery with extracts to the regional pollen spectrum, for effective immunotherapy. It can also be helpful for patients in taking prophylactic measures such as the planning of outdoor activities, contributing to an improvement on their life quality.

In Portugal, some studies have been conducted on the type and concentration of airborne pollen grains in the atmosphere of several cities[1-5] as well as its relationship with meteorological factors[6]. Also, the classification of allergenic pollen types, through immunological studies[7,8] associating the regional pollen exposure with allergic respiratory hospital admissions[7], has been performed.

Pollen allergy has a remarkable clinical impact all over Europe, and there is considerable evidence suggesting that respiratory pollen allergic diseases are increasing in both prevalence and severity in most industrialized and developing countries[9].

The plants of Urticaceae family that comprise the genus *Parietaria* are dicotyledonous weeds and have been described as the most widespread allergenic plants in the Mediterranean area[10]. Its pollen is responsible for many cases of severe pollinosis mainly due to its high pollen output and the long persistence in the atmosphere[10,11].

Parietaria judaica and *Parietaria officinalis* are the most common allergenic species of this genus and are highly cross-reactive[12]. *Parietaria judaica* or wall pellitory (Figures 1a, 1b) has very strong allergenic properties having a particular allergological interest[13]. It is a sprawling, many-branched, bushy perennial weed, with delicate reddish stems. It is wind-pollinated and grows spontaneously on neglected areas, along roadsides, on walls, inside riverside forests and hedges, reaching from 30 to 100 cm in high. The leaves are 3 to 12 cm long and oval in shape, with hairs on the veins on the lower surface. The inconspicuous green stalkless flowers are clustered in the leaf axils.

P. judaica pollen contains at least nine allergens identified of which two major allergens – Par j 1 of 14.7 kDa and Par j 2 of 11.3 kDa that have already been biochemically characterized and molecular cloned by different immunochemical methodologies[14,15,16]. They were reported to belong to a family of glycoproteins known as non-specific lipid transfer proteins (LTP)[13,17,18]. Their recombinant proteins showed a prevalence of 95% and 82% among *P. judaica*-sensitive patients[19,20]. The sequence analysis of the cDNA expression library has shown that Par j 1 allergen (Par j 1.0102) contains 176 amino acids with an amino-terminal region of 37 amino acids and a mature processed protein of 139 amino acids with a molecular weight of 14,729 kDa[19]. The other major allergen of *P. judaica* pollen, Par j 2 (Par j 2.0101), was also sequenced and consists of a polypeptide of 133 amino acids with an amino-terminal signal peptide of 31 amino acids giving a mature processed protein of 11,344 kDa[20]. More recently, a profilin of *P. judaica* – Par j 3 – was identified as having a molecular mass similar to Par j 1 and Par j 2, within the range of 14 kDa[21]. In our investigation, we sought to study the aerobiology and allergenic proteins of *Parietaria judaica* pollen collected in the Porto region.

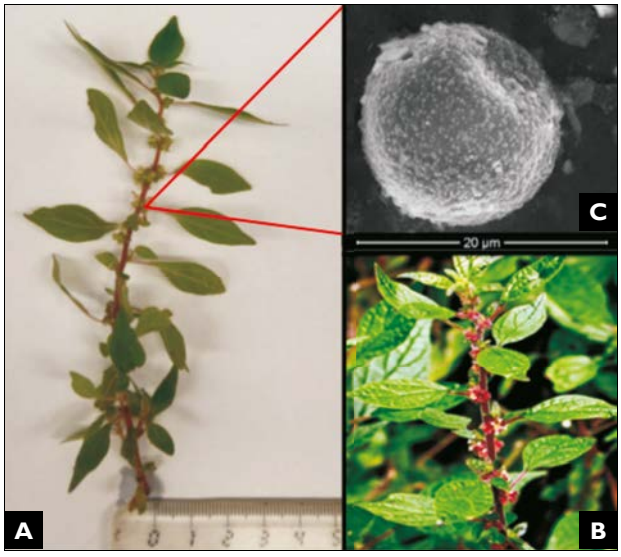

Figure 1. *Parietaria judaica* (wall-pellitory). Plant with dehiscent anthers (A and B). Pollen grain surface at scanning electron microscopy (C)

MATERIAL AND METHODS

Study area

This study was conducted in the Porto region, the second largest Portuguese city, located in the Northwest of Portugal (41°11' N, 8°39'W) being limited by the Atlantic Ocean and the Douro River. It presents a Mediterranean climate according to the Köppen climate classification, however, an Atlantic influence can be observed. Temperature is mild; January is the coldest month and July the hottest. The average minimum temperature is 10±0.7°C and the average maximum 19±0.6°C. Annual mean relative humidity ranges between 75 and 80%, and rainfall is mainly concentrated in winter and spring[22].

Aerobiological monitoring

Airborne pollen monitoring was continuously performed from 2003 to 2012, using a 7-day Hirst-type volumetric trap (Burkard Manufacturing Co., U.K.) set on the roof of the Faculty of Sciences in Porto, 20 m above ground level, and calibrated to sample air at 10 L per minute. Pollen grains were trapped on a Melinex tape coated with silicone oil, which was then cut into daily segments and mounted on slides with a mounting media of glycerol jelly with fuchsine. The daily and hourly mean concentration of *Parietaria* spp. pollen was estimated using an optical microscope (DMLB, Leica) with x400 magnification along 4 full lengthwise traverses. Pollen counts were expressed as the sum of the number of pollen grains per cubic meter of air for a 24-hour period. *P. judaica* pollen is oblate (circular) usually tri-zonoporate and its polar axis commonly ranges from 12 to 16 µm. The external layer named exine is thin, psilate to scabrate with spinulas uniform and densely distributed over the surface (Figure 1c).

The intradiurnal airborne pollen concentration was determined, for each year, from the hourly values of atmospheric concentration registered during the main pollen season[23]. For this, the days when atmospheric pollen concentrations were superior to the third quartile of the main pollen season concentration and no precipitation occurred were selected. Since the hourly counts vary widely interdaily and interannually, the values were expressed in percentage.

The main pollen season was defined using a non-linear logistic regression model fitted to the values of the accumulated sum of the daily airborne pollen concentration. After adjusting the model to each year, a one-sided t-test was used at the 5% level, in order to estimate the beginning and ending dates of the main pollen season. These dates correspond to the thresholds where the daily difference between the pollen emission model and its superior and inferior asymptotes were significant[24].

Pollen data did not present normal distribution, so data was transformed using Log (*yi*+1), where *yi* corresponds to each pollen value registered, once values were very dispersed and some were null. The relationship between the main meteorological parameters (mean, minimum and maximum temperature and relative humidity) and the daily pollen concentration was calculated using a Pearson correlation coefficient.

Sensitization data

Data from allergic patients' sera presenting specific IgE levels to pollen extracts, performed by ImmunoCAP™ FEIA test (Phadia AB), were obtained from a central hospital in Porto region between 2007 and 2012. This study was performed only with anonymous files of sensitization test results due to confidential policies and therefore information regarding gender, sex or age was unavailable.

Patients presenting specific IgE levels to *Parietaria judaica* (w21) or *Parietaria officinalis* (w19) and both pollen extracts were accounted by different levels of specific IgE recognition: 0.35-0.7 kU_A/L; 0.7-3.5 kU_A/L; 3.5-17.5 kU_A/L; 17.5-50 kU_A/L; 50-100 kU_A/L; >100 kU_A/L.

Biochemical and Immunological studies

Pollen samples

Anthers were collected during *Parietaria judaica* flowering season and after removal of other plant parts, they were dried at 27°C, gently crushed and the pollen thus released was passed through different grades of sieves to obtain pure pollen. Pollen samples were then stored at –20°C.

Patient sera

Twenty-one random *Parietaria*-sensitive patients with clinical history of respiratory allergy and presenting a positive response to skin–prick tests (SPT) using *Parietaria judaica* pollen extracts were chosen. A positive SPT was considered when allergen ring diameter was 3 mm or greater after subtraction of the negative control, recorded at 15 minutes post-injection. Histamine (10 mg/mL) and saline solutions of glycerin were used as the positive and negative controls, respectively. Blood from those patients was collected by venipuncture, sera were separated from the whole blood and allergen specific IgE levels were measured by ImmunoCAP™ FEIA test (Phadia). The chosen sera had specific IgE levels against *Parietaria judaica* pollen extracts (w21) between 0.35 and >100 kU_A/L. One serum from a patient with low total IgE levels and negative to *Parietaria judaica* pollen was also used as a negative control (NC). This study was performed only with anonymous files due to confidential policies and therefore information regarding gender, sex or age was unavailable.

Protein extraction and quantification

P. judaica dry pollen was suspended in 1:20 (w/v) phosphate saline buffer at pH 7.4 at 4°C. Soluble proteins were extracted in the same buffer by continuous stirring for 4 h. The suspension was then centrifuged at 13,200 rpm for 30 min at 4°C. The supernatant was filtered through a nitrocellulose 0.45 µm Millipore syringe filter and centrifuged once again. The soluble protein content of crude pollen extracts was colorimetrically quantified using the Coomassie Protein Assay Reagent.

SDS-Polyacrylamide Gel Electrophoresis and staining

SDS-PAGE was performed in 12.5 % polyacrylamide gels under reducing conditions. The polypeptides of pollen crude extracts were visualized by Coomassie Brilliant Blue R-250 staining. The molecular weights of protein bands were estimated by comparison with a protein marker (Fermentas now Thermo Scientific). The antigenic profile was studied using a Molecular Imager GS800 calibrated densitometer and Quantity One 1-D Analysis, v 4.6 software (Bio-Rad Laboratories).

Glycosylated protein detection

Total carbohydrate detection of *P. judaica* protein extract transferred to a nitrocellulose membrane was performed using an ECL™ Glycoprotein Detection System RPN2190 (GE Healthcare, Amersham), following the manufacturer's instructions. This system allows labeling of a carbohydrate moiety with biotin through the periodate oxidation step and later detection with streptavidin-HRP and enhanced chemiluminescence.

Immunoblot and inhibition experiments

Protein bands separated by SDS-PAGE were electrophoretically transferred on a blot apparatus (TE22 Mighty Small Transfer Unit, GE Healthcare, Amersham) to a nitrocellulose membrane (Protran, Whatman® Schleicher and Schuell, Germany). Transfers were carried out in a solution of 192 mM glycine, 25 mM Tris and 20% methanol during 2 h at 200 mA. The membranes were saturated during 1 h with 5% non-fat dry milk, 0.1% goat serum in TBS-T (20 mM Tris, 150 mM NaCl with 0.1% Tween 20) and incubated overnight at 4 °C with *P. judaica* allergic patient sera (1:10); polyclonal serum raised against recombinant maize profilin (ZmPRO3) (1:4000), expressed in *E. coli* and produced in a New Zealand white rabbit[25]; Lol p 1 (1:200) or Par j 1/Par j 2 (1:100), used as reference material, purified from rye grass pollen extract

by a combination of ion exchange chromatography and gel filtration[26]. After washing, blots were probed with mouse anti–human IgE-HRP or with goat anti-rabbit IgG--HRP during 1 h at 4.°C. The reaction was revealed using peroxidase ECL substrate and exposed to ECL-Hyperfilm (GE-Healthcare). The chemiluminescent signal was exposed to AGFA medical X-ray film, developed by Fuji medical film processor model FPM 100A, and the antigenic profile was quantified as described above.

SDS-PAGE resolved *P. judaica* pollen proteins were transferred onto nitrocellulose membranes. After blocking, two blots were kept overnight at 4.°C with aliquots of patient serum only sensitized to *P. judaica* pollen extract with saturating buffer as the control experiment (1:5 v/v) or pre-incubated with *P. judaica* pollen extract as inhibitor. Bound IgE was detected as described previously.

RESULTS

Aerobiological monitoring

Considering the period between January 2003 and September 2012, *Parietaria* spp. pollen was the most abundant type in the atmosphere of Porto and constitutes nearly 17% of the total pollen spectrum. The annual pollen concentration ranged from 654 pollen grains in 2003 to 2,921 pollen grains per cubic meter of air in 2007 (Table 1).

The highest *Parietaria* spp. airborne pollen concentrations were mainly found between April and August, being the maximum airborne concentration usually between May and July. The average main pollen season was of 13±4.3 weeks, being the longest main pollen season recorded in 2004, with 153 days and the shortest in 2012, with 62 days. The average airborne pollen peak levels attained within a 24 h-period was 50 pollen.m^{-3} of air, being the highest values observed in 2007, with 110 pollen.m^{-3} of air, and the lowest in 2003, with 18 pollen.m^{-3} of air (Table 1; Figure 2A).

Concerning intradiurnal variation, the highest airborne pollen concentrations were recorded during the morning period. *Parietaria* airborne pollen started to increase at first day hours (7 a.m.) reaching a maximum value between 9-11 a.m. that corresponded on average to 10% of its daily total. A similar concentration, around 3%, during the other hours of the day was observed (Figure 2B).

Overall correlations between *Parietaria* spp. airborne pollen concentration and the meteorological factors showed positive correlations with the thermal parameters and negative with precipitation and relative humidity. Consistent correlations along the studied years were observed for maximum and mean temperature (Table 2).

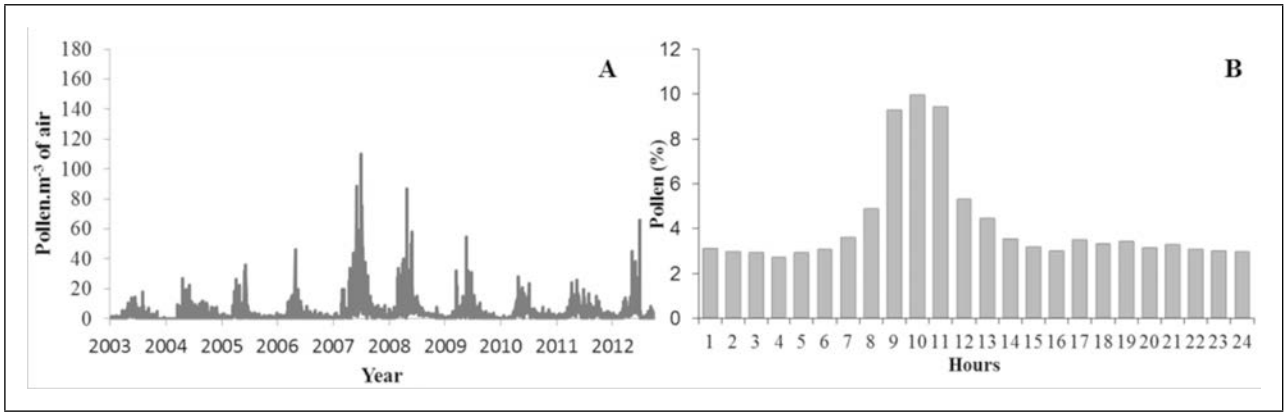

Figure 2. *Parietaria* spp. daily airborne pollen distribution (A) and airborne average hourly distribution, expressed in percentage (B) in Porto city from January 2003 to September 2012

Table 1. Main pollen season (MPS) characteristics of Parietaria spp. in the city of Porto from 2003 to 2012

	MPS (Julian days)				Pollen	
	x_i	Peak	x_s	D	Annual total	Peak (pollen.m^{-3} air)
2003	121	168	216	95	654	18
2004	125	201	278	153	948	27
2005	101	132	164	63	952	36
2006	90	125	160	70	810	46
2007	133	173	214	81	2921	110
2008	90	128	167	77	2360	87
2009	107	152	198	91	1401	55
2010	120	157	195	75	1107	28
2011	103	171	239	136	1417	26
2012	110	140	172	62	1399	66
Average	110	155	200	90	1397	50
CV (%)	13	16	1 9	34	51	60

x_i – beginning date; peak – peak date; x_s – end date; D – duration; CV – coefficient of variation.

Table 2. Pearson correlation coefficients between *Parietaria* spp. airborne pollen concentration and the meteorological factors in Porto (2003-2012)

	R (mm)	Temperature (°C)			Relative humidity (%)		
		Max	Min	Mean	Max	Min	Mean
2003	**-0.304*****	**0.490*****	**0.266*****	**0.438*****	**-0.287*****	**-0.444*****	**-0.433*****
2004	-0.034	0.129	-0.106	0.027	0.017	**-0.181****	-0.122
2005	-0.051	**0.335*****	**0.301****	**0.374*****	-0.135	-0.142	-0.233
2006	-0.062	0.042	0.129	0.019	-0.009	0.105	0.052
2007	-0.075	0.120	0.072	0.109	-0.12	-0.095	-0.13
2009	-0.135	**0.413*****	**0.247****	**0.347*****	**-0.281*****	**-0.288*****	**-0.293*****
2010	-0.155	**0.372*****	0.196	**0.305*****	**-0.268****	**-0.233****	**-0.290*****
2011	-0.067	**0.279*****	0.056	**0.239*****	**-0.290*****	**-0.311*****	**-0.320*****
2012	-0.128	**0.470*****	**0.386*****	**0.483*****	0.082	-0.237	-0.102
Overall (2003-2012)	**-0.145*****	**0.398*****	**0.387*****	**0.415*****	**-0.104*****	**-0.143*****	**-0.162*****

** Significant correlation at the 95% probability level; *** significant correlation at the 99% probability level; R – precipitation; max – maximum; min – minimum.

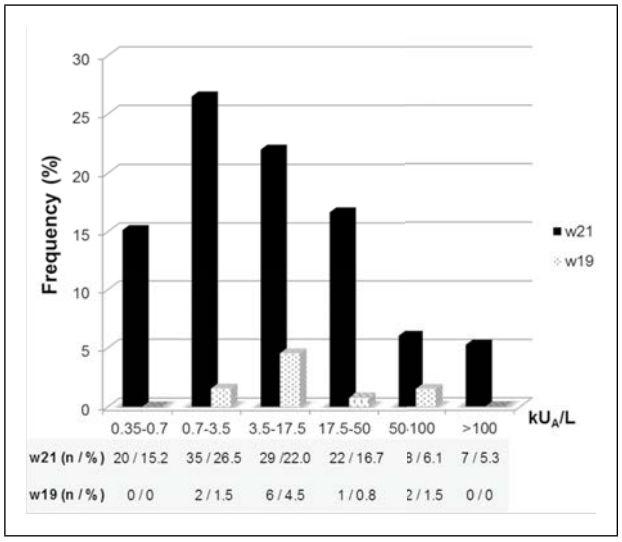

Figure 3. Frequency of sensitization to *P. judaica* (w21) or *P. officinalis* (w19) pollen extracts. Six specific IgE levels (kUA/L): 0.35-0.7; 0.7-3.5; 3.5-17.5; 17.5-50; 50-100 and >100. n: represents the number of sensitized individuals among the 132 patients sensitized

Sensitization assessment

Out of 415 patients presenting specific IgE levels to any pollen, previously evaluated by SPT and presenting positive reaction to pollen extracts, 142 patients (34.2%) presented specific IgE levels to *Parietaria spp.* pollen extracts. Among these, 84.5% (n=120) were only positive to *Parietaria judaica* (w21), 8.5% (n=12) to *Parietaria officinalis* (w19) and 7.0% (n=10) to both pollen types (w21 and w19).

Among the specific IgE levels, the highest percentage of patients had 0.7-3.5 kU_A/L for w21 (Figure 3). Extreme IgE affinity (>100 kU_A/L) was only achieved by *P. judaica* sensitized patients. Demographic data were not available due to confidentiality policy.

Immunoreactivity of pollen extracts, inhibition experiment

SDS-PAGE protein profiles of *P. judaica* pollen extracts are presented in Figure 4. The resolved protein fractions on SDS-PAGE ranged from 100 to 10 kDa, being the most noticeable bands around 55, 45, 36, 30, 22, 18, 14, 12 and 11 kDa.

The reactivity of the protein bands from *P. judaica* pollen extracts was assayed by immunoblotting. The specific IgE binding fractions incubated with *P. judaica* sensitive patients' sera are shown in Figure 4. Among the twenty--one tested patient sera, four main groups of common reactive bands were observed: one band around 55 kDa (n=15, 66.7%), one group with two bands of 36 and 30

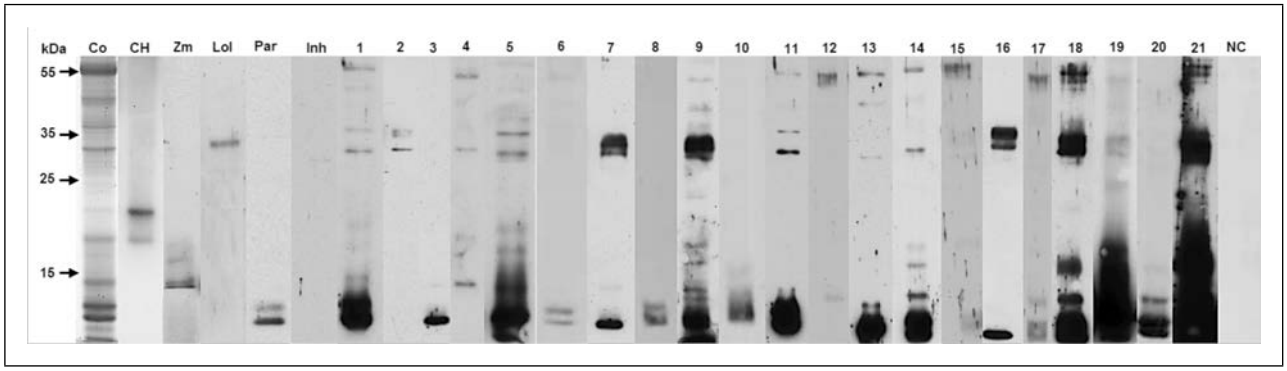

Figure 4. Polypeptide and immunoblot results of *P. judaica* pollen extracts. Coomassie blue staining of pollen crude extracts (Co) and glycosylated proteins of whole extract (CH). Blots were probed with antibodies namely ZmPRO3 (Zm), Lol p 1 (Lol) and Par j 1/Par j 2 (Par). An inhibition experiment was performed with *P. judaica* protein strip incubated with serum pretreated with *P. judaica* pollen extract as inhibitor (Inh). Immunoblots (1-21) probed with escalating IgE values (0.35 to >100 uKA/L) from *P. judaica* allergic patients sera. A non-sensitive pollen serum to *P. judaica* was used as a negative control (NC). Numbers on the left indicate the molecular mass markers

kDa (n=13, 61.9%), another group of two bands with 22 and 18 kDa (n=8, 38.1%) and a fourth group composed by three bands of 14 kDa (n=14, 66.7%) and a pair of 12 and 11 kDa (n=18, 85.7%).

To ensure that a serum is specifically sensitive to *Parietaria judaica* pollen extracts an inhibition experiment was conducted. Pre-incubation of *P. judaica* pollen protein extracts with a serum sensitized to *P. judaica* pollen inhibited completely the patient serum sensitive to *P. judaica*.

Allergen-like proteins and glycosylated protein detection

To identify Par j1/Par j2-like proteins in *P. judaica* pollen extracts it was also carried out an anti-Par j 1/Par j 2 immunoblot, which revealed two protein bands of 12 and 11 kDa.

To analyze the similarity of *P. judaica* pollen proteins to some relevant allergens of non-taxonomically-related proteins an immunoblot using ZmPRO3, a profilin from *Zea mays*, and Lol p 1, a major grass pollen allergen of *Lolium perenne* were performed. After the incubation with ZmPRO3 and Lol p 1, *P. judaica* immunoblots revealed one protein band around 14 kDa and another band around 32 kDa, respectively.

We also investigated the presence of glycosylated proteins on *P. judaica* pollen extracts. After total carbohydrate labeling, two bands were present in *P. judaica* extract profile, one around 22 kDa and another of around 18 kDa.

DISCUSSION

The weed *Parietaria* spp., mainly *P. judaica*, is considered one of the most relevant airborne allergens of the Mediterranean area. So, the present study performed aerobiological monitoring in a Northern Portuguese coastal city to assess the IgE affinity to *P. judaica* pollen protein extracts of sensitized individuals.

Atmospheric pollen concentration varies from region to region, depending on its geographic characteristics as different climatic areas present different vegetation. In the Porto region, *Parietaria* spp. is the most abundant pollen present in the atmosphere. The same pattern was also found in Lisbon (Portugal's capital), but was different from Coimbra, Évora and Portimão where Urticaceae that includes *Urtica* spp. and *Parietaria* spp. are the fourth most representative pollen types[1]. This pollen is present all year long in the atmosphere of Porto; this can be related to the fact that *Parietaria* spp. is a weed species that is able to grow and flower along the year, and also presents light and aerodynamically shaped pollen (small size 12-16 µm) that can resuspend in the air very easily. Consequently, airborne pollen can be found in the atmosphere outside of the main pollen emission season. This sustains several implications in allergenic sensitization, observed mainly during the spring-summer months. In fact, some days presented airborne pollen levels of *Parietaria* spp. superior to 30 pollen grains.m^{-3} per day and in some years even superior to 60 pollen grains.m^{-3} per day that are considered, according to the Portuguese Aerobiology Network, moderate and high risk for allergenic reactions.

The high *Parietaria* spp. airborne pollen concentrations are found during the sunlight hours. This pattern is common to a wide range of pollen types[4], since the anthesis and pollen dispersion occurs mainly during daylight. Likewise, the increase in pollen concentration during the first hours of sunlight coincides with a period of thermal inversion, increase in wind velocity and decrease in relative humidity that facilitates anther dehiscence, pollen emission and dispersion[27]. Apart from the high airborne pollen peak reported at 10h, *Parietaria* spp. pollen was presented within an almost constant threshold during the other hours of the day. This fact is important because it can contribute to the exacerbation of the allergic symptoms due to the cross-reactivity with other pollen types also present in high concentrations in the atmosphere.

Consistent correlations between airborne pollen concentration and the meteorological parameters were only

observed for temperature. This is in accordance with the common knowledge that the heat parameters have a positive influence on airborne pollen concentration, since temperature favors pollen release. Regardless of non-significant, the influence of precipitation was always negative and its effect is associated with atmosphere washing, which can have a positive effect on decreasing pollen allergy outbreaks.

In the present study, we evaluated the prevalence of sensitization to *Parietaria* spp. among 415 pollen sensitive individuals based on the IgE serum levels measured. About 34.2% had specific IgE to *Parietaria* spp. from which 84.5% specifically and only to *P. judaica*. This pollen species also seems to be the only weed able to induce extreme IgE recognition in all pollen sensitive patients studied (data not shown). Sensitization, evaluated through skin-prick tests, to this pollen extract has also been described in several studies in other Mediterranean areas. In Central Greece, it was reported a prevalence of 10.3%[28]; Gioulekas et al.[29] reported 15.3% in Northern Greece. In Spain, it was verified that 14% of the population with respiratory allergy symptoms was sensitized to *P. judaica* pollen[30], and in Genoa (Italy) among 6800 patients 70.6% showed positive SPT to *P. judaica* pollen extracts[31]. In a sensitization study performed in East-central region of Portugal, the authors sought to evaluate aeroallergens sensitizations among individuals suffering from respiratory allergy according to the environment they lived in (urban vs. rural exposure). These authors verified that among the 1096 pollen positive SPT assessed, 29.4% living in urban areas were sensitized to *P. judaica* pollen contrasting to 14% living in a rural environment[32].

In our study, by using a panel of 21 *P. judaica* sensitive IgE sera, we observed that protein bands around 55 kDa, 36-30 kDa, 14 kDa and 12-11 kDa were preferentially recognized. It was verified that the majority of the 21 IgE sera tested (85.7%) recognized a pair of proteins with 12-11 kDa that may correspond to the specific allergens of *P. judaica* pollen extracts[13]. An anti-Par j 1/Par j 2 was used to probe *P. judaica* whole extracts and the two protein bands of low molecular weight revealed can be the major allergens of Par j 1/Par j 2-like proteins capable of inhibiting up to 95% of the total IgE binding activity of *P. judaica* pollen extracts[33].

Furthermore, we identified two glycosylated proteins around 22 and 18 kDa from *P. judaica* whole pollen extracts, which do not correspond to the most IgE reactive group of protein bands. Regarding the estimated molecular weights, these glycosylated proteins may be related to allergens around the same molecular weight recognized by 38.1% of the 21 IgE patient sera tested.

By using rabbit polyclonal IgG ZmPRO3 antibody as a probe instead of IgE sera, a protein band of around 14 kDa was recognized and may correspond to a profilin-like protein, a well-know panallergen that has already been described in *Parietaria judaica* pollen extracts[21]. In a skin-prick test sensitization study, it was reported that 64.1 % of profilin-sensitive patients were also sensitized to *P. judaica* pollen, suggesting that this panallergen may share determinant epitopes with a 14 kDa *P. judaica* pollen protein[34]. Asturias et al.[21] showed a very low content *P. judaica* profilin when compared to non-related pollen extracts. In our study, 61.9% of IgE sera sensitive to *P. judaica* reacted to a protein band around 14 kDa that may well correspond to *P. judaica* profilin.

Regarding other possible cross-reactive proteins, after probing blots with Lol p 1, a group 1 grass allergen, it was identified a single band of 32 kDa that may correspond to an expansin-like protein[35] in *P. judaica* pollen extracts and can be related with the pair of 36-30 kDa identified with the IgE sera sensitive to *P. judaica.*

CONCLUSIONS

Parietaria spp. is the most abundant pollen type in the atmosphere of Porto, presenting a main pollen season between April and August and attaining airborne levels considered to be moderate and high risk for allergenic reactions. Its airborne pollen concentration is highest at

the first sunlight hours and it is positively correlated with ambient temperature.

P. judaica in particular sensitizes a considerable fraction of pollen-sensitive individuals and several reactive protein bands were recognized by *P. judaica* sensitive IgE sera, which included a pair of proteins with 12-11 kDa that may correspond to the specific allergens of *P. judaica* pollen extracts, glycosylated proteins of 22 and 18 kDa and a few cross-reactive proteins of 14 kDa and 32 kDa, which may correspond to a profilin-like and an expansin-like proteins, respectively. So, *P. judaica* pollen is an important source of weed-sensitization in Porto region.

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