



Sensitization to subtropical grass pollens in patients with seasonal allergic rhinitis from Bahia Blanca, Argentina

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ABSTRACT

Background: Grass pollinosis is an important contributor to allergic diseases, with varying patterns and frequency of allergens according to the geographical location studied. Our study aims to provide a better understanding of subtropical grass pollinosis in Argentinian patients with seasonal allergic rhinitis.

Methods: We conducted a retrospective cross-sectional study involving 894 patients with seasonal allergic rhinitis from Bahía Blanca, Argentina. Skin prick tests were performed to selected pollen species belonging to three subfamilies of the *Poaceae* genera. Frequency of sensitization to specific grass pollen extracts, cross-reactivity of allergens assessed by skin prick test, and possible associations between allergen extracts and asthma or allergic conjunctivitis were analyzed.

Results: Sensitization to the *Pooideae* subfamily was the most frequent, encompassing 86.8% (CI: 84.4%–88.9%) of the studied population. Positive reactions to allergen extracts from the *Chloridoideae* and the *Panicoideae* subfamilies showed smaller papule size than allergen extracts from the *Pooideae* subfamily ($\chi^2(5) = 83.75, p < 0.001$). Patients with a positive skin prick test (SPT) to a specific extract were more likely to present some degree of cross-reactivity to the remaining pollens when compared to patients with negative SPT to the same specific extract. Even though the proportion of patients presenting with asthma (46.9%) was higher than those with conjunctivitis (22.6%), there was only a statistically significant association between sensitization to *Festuca arundinacea* ($\phi = 0.089, p = .009$), *Phalaris arundinacea* ($\phi = 0.074, p = .032$) and *Paspalum notatum* ($\phi = 0.070, p = .038$) and the presence of conjunctivitis.

Conclusions: Our results suggest a high frequency of sensitization to grass pollen extracts from the *Poaceae* family among patients with seasonal allergic rhinitis. Overall, sensitization to the *Pooidae* subfamily was the most common, where *Phalaris arundinacea* presented the highest frequency.

Keywords: Allergic rhinitis, Pollen allergy, Poaceae, Gramineae, Latin America

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BACKGROUND

Grass pollinosis is an important contributor to allergic diseases, with varying patterns and frequency of allergens according to the geographical location studied.¹ In general, there is an inverse distribution of temperate and subtropical grasses, with species of the latter being more abundant the closer to the equator.² More than 95% of allergy-relevant grass species belong to three subfamilies of the *Poaceae* family: *Pooideae*, *Chloridoideae*, and *Panicoideae*.³ *Pooideae* grasses are most frequent on temperate climate zones, while *Chloridoideae* can be found on temperate and sub-tropical grasslands, and *Panicoideae* is mainly found on subtropical grasslands.³

Identification of sensitization patterns and airborne allergen counts are clinically relevant to provide a more accurate diagnosis in allergic conditions such as seasonal allergic rhinitis and have the potential for treatment through immunotherapy.¹ However, climate change as a result of global warming is altering the distribution, timing, and allergenicity of grass pollens, with an expected increase of subtropical grasses.⁴ Furthermore, with the widening of subtropical climate zones, and the increasing population size living in these regions, it is expected that allergy to subtropical grasses will play a key role in the exacerbation of allergic diseases in the future.^{5,6}

Much of what is known about the allergenic components of grasses, distribution, and patterns of sensitization primarily comes from developed regions, where temperate grass pollinosis is more prevalent.³ Data from Latin America in this regard are relatively sparse. In Argentina, there is a characteristic geographic distribution and high-frequency airborne count of pollens.⁷ However, to the best of our knowledge, there are no studies that characterize the frequency of sensitization to specific extracts. Our study aims to provide a better understanding of subtropical grass pollinosis in Argentinian patients with seasonal allergic rhinitis, by identifying the frequency of sensitization to specific grass pollen extracts, cross-reactivity of allergens assessed by skin prick test, and the possible associations between allergen extracts and asthma or allergic conjunctivitis.

METHODS

Study design and procedure

We conducted a retrospective cross-sectional study involving 894 patients with seasonal allergic rhinitis from Bahía Blanca, Argentina (Table 1). Participation was restricted to patients presenting symptoms for at least two consecutive years, with at least one positive skin prick test (SPT) to any of the six allergen extracts of the *Poaceae* genera studied and had resided at the city of Bahía Blanca for at least five years. Skin prick tests (SPTs) were performed at the *Instituto de Alergia e Inmunología del Sur* (Bahía Blanca, Buenos Aires, Argentina) from November 2010 to November 2016 using GREER® GreerPick™ Skin Testing Devices. Allergen extracts were provided by *Alergo Pharma SRL* and produced by *Greer Labs (GREER®)*.

Skin prick tests were considered positive when the papule diameter was equal to or greater than 3 mm from the negative control papule.⁸ The selected pollen species belonged to three subfamilies of the *Poaceae* genera: the *Pooideae* subfamily (*Lolium perenne*, *Festuca arundinacea* and *Phalaris arundinacea*), the *Panicoideae* subfamily (*Paspalum notatum* and *Sorghum halepense*) and the *Chloridoideae* subfamily (*Cynodon dactylon*) (Table 2). Species were selected due to their representativeness of the area of study, except for *Paspalum notatum*, which was selected due to its taxonomic affinity to *Paspalum quadrifarium*.^{9,10} Even though *Paspalum quadrifarium* is the regional representative specie, there was no extract available for testing.

Characteristics	Value n (%)
Age (mean)	32.2 (17.0)
Gender	
Male	457 (51.1)
Female	437 (48.9)
Asthma	419 (46.9)
Allergic conjunctivitis	202 (22.6)

Table 1. Demographic and clinical information of surveyed population (n = 894)

Species	Subfamily	Status ^a	Native relatives
<i>Lolium perenne</i>	Pooideae	Exotic	N/A
<i>Festuca arundinacea</i> ^b	Pooideae	Exotic	<i>F. pampeana</i> <i>F. ventanicola</i>
<i>Phalaris arundinacea</i>	Pooideae	Exotic	<i>P. platensis</i>
<i>Cynodon dactylon</i>	Chloridoideae	Exotic	N/A
<i>Paspalum notatum</i>	Panicoideae	Native	<i>P. quadrifarium</i> ^c <i>P. dilatatum</i>
<i>Sorghum halepense</i>	Panicoideae	Exotic	N/A

Table 2. Species used in this study and related species native from the region of Bahía Blanca. Notes: Species names correspond to the updated nomenclature from the Flora del Conosur Database (IBODA). N/A, not applicable. a. The term 'Status' refers to evolutive origin in botany. b. *Festuca arundinacea* was used instead of the old name *Festuca elatior*. c. Allergen extracts not available

Ethical considerations

This study was approved by the ethics committee: Comité de ética e Investigación en Seres Humanos (CEISH), in accordance to the principles established by the declaration of Helsinki.

Statistical analyses

Throughout data analyses, SPT was manipulated either as a continuous (papule's diameter in mm) or dichotomic (positive or negative) variable. Analyses treating SPT as a continuous variable were intended to evaluate the degree of reactivity upon a certain dependent variable, whilst tests analyzing SPT as a dichotomic variable were included to give a better clinical utility.

Descriptive statistics for proportions of patients with a positive SPT for the selected species were represented using intervals of 95% confidence. Cochran's Q test was used to determine the statistical significance of such proportions. A Cochran's Q test was also performed considering the SPT response to pollen extracts by subfamilies. Difference of papule's size between allergen extracts were analyzed using Friedman test.

Thereafter, a Kendall's tau b to assess the association between the degree of reactivity of each extract and the SPT response of the other species was performed. A Chi-square test for association was reproduced as well, for the same purpose. We employed the same tests to determine the

association between SPT to the allergen extracts and the presence of asthma and allergic conjunctivitis.

All the data was analyzed using SPSS, version 24.0 software (SPSS Inc., Chicago, IL, USA). We performed Fisher's exact test were necessary. A p-value of less than 0.05 was considered statistically significant for all tests.

RESULTS

Sensitization to the *Pooideae* subfamily was the most frequent, 86.8% (CI: 84.4%-88.9%) of the studied population, compared to the other subfamilies: the *Panicoideae* species disclosed a 69.6% (CI: 66.5%-72.5%) positive while the *Chloridoideae* subfamily presented a 48.1% (CI: 44.8%-54.1%) positive. The Cochran's Q test suggested that the prevalence in the three groups was different ($\chi^2(3) = 319.11$, $p < .01$). The same analysis, considering species independently of their subfamilies, was statistically significant as well ($\chi^2(5) = 69.31$, $p < .001$). Descriptive statistics of allergy prevalence by species from the three subfamilies are best depicted in Fig. 1 and Supplemental Appendix Table S1.

Mean difference and distribution

Positive reactions to allergens from the *Chloridoideae* and the *Panicoideae* subfamilies showed lower reactivity than allergens from the *Pooideae* subfamily. Differences in the sizes of papule after

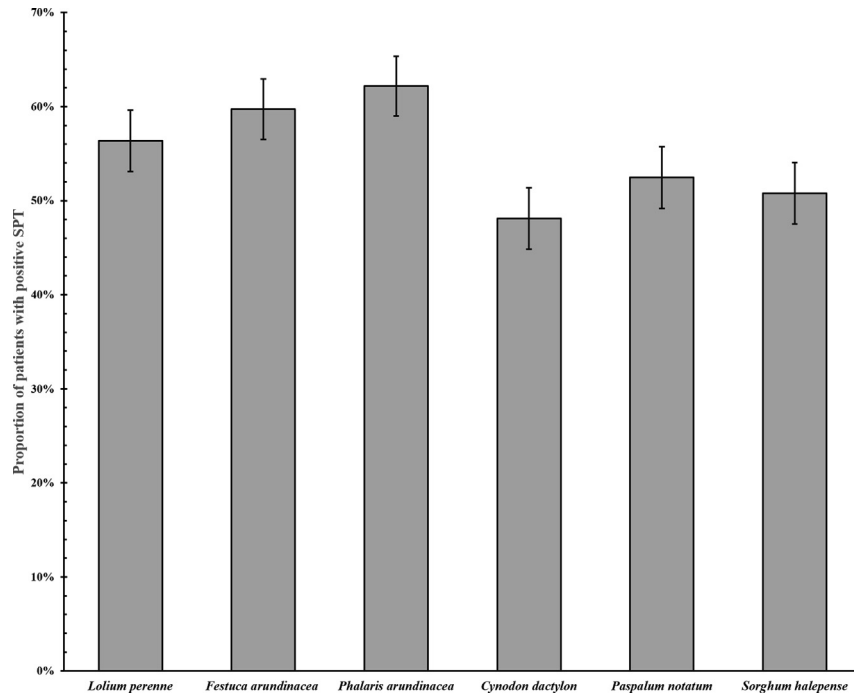


Fig. 1 Prevalence of sensitization to grass pollen allergens in patients with seasonal allergic rhinitis. Values shown are mean \pm SE in a percentage scale

the allergen extract administration were statistically significant ($\chi^2(5) = 83.75, p < .001$). The distribution of the measures of the SPT of every allergen extract are depicted in Fig. 2. Notice that all allergen extracts but *Festuca arundinacea* and *Phalaris arundinacea*, showed a longer segment between the third quartile and the median than the segment within the median and first quartile. This description suggests a relatively uniform distribution for all species except for *Festuca*

arundinacea and *Phalaris arundinacea*, which were left-skewed. Although the prevalence to the *Pooideae* subfamily of pollens seemed to be higher, the sensitivity of positive cases to the *Panicoideae* and the *Chloridoideae* subfamilies exhibited more outliers.

Correlations cross-reactivity

SPT for each of the allergen extract reported cross-reactivity between all species, as assessed by

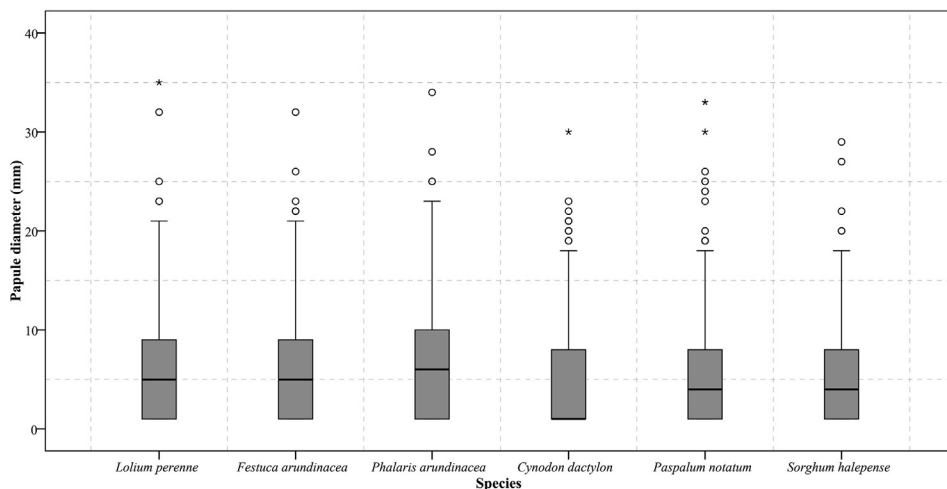


Fig. 2 Papule size distribution by pollen grass species. Median (midline), box (25th and 75th percentiles), whiskers (extrema), \circ (outlier) and * (extreme value)

Kendall's τ_b . The most negligible correlation was appreciated between *Cynodon dactylon* and *Phalaris arundinacea* ($\tau_b = 0.160$, $p = p < .001$), while the best positive correlation was measured between *Phalaris arundinacea* and *Festuca arundinacea* ($\tau_b = 0.330$, $p < .001$), although still low. Moreover, Chi-squared tests revealed similar results, denoting that patients with positive SPT to any allergen extract were more likely to have cross-reactivity to all the other extracts when compared to patients with a negative SPT to the same specific allergen extract. Proportions of patients presenting cross-reactivity and the correlation strength assessed by Phi coefficient are best portrayed in Fig. 3.

Overall, the proportion of patients also presenting with asthma was higher in the negative SPT group, except for *Paspalum notatum*. On the other hand, the proportion of patients with conjunctivitis was higher in the positive SPT group (Fig. 4). However, Kendall's τ_b revealed no correlation between the degree of reactivity to pollen extracts and asthma, whilst correlations for allergic conjunctivitis were significant for *Festuca arundinacea* ($\tau_b = 0.088$, $p = .003$) and *Phalaris arundinacea* ($\tau_b = 0.066$, $p = .025$). Analyzing the SPT as a nominal variable, Chi-squared tests for

association did not reveal a statistically significant correlation for asthma. With respect to conjunctivitis, there was a positive relationship for *Festuca arundinacea* ($\phi = 0.089$, $p = .009$), *Phalaris arundinacea* ($\phi = 0.074$, $p = .032$) and *Paspalum notatum* ($\phi = 0.070$, $p = .038$), though, Phi coefficient identified it as negligible.

DISCUSSION

Data on sensitization to grass pollen extracts primarily comes from developed regions such as North America and Europe, with overall sensitization rates around 15-20% among the general population.³ However, these rates tend to vary depending on the geographical location and specific sample analyzed. In our study, we found patients with seasonal allergic rhinitis to be most frequently sensitized to extracts belonging to the *Pooideae* subfamily (86.8%), followed by *Panicoideae* (69%), and *Chloridoideae* (48.1%). Although data from Latin America in this regard is relatively sparse, our results suggest that there is a higher frequency of sensitization to grass allergens when compared to the rate of sensitization found on the general population from other countries of the region (20-30%).¹¹⁻¹⁴

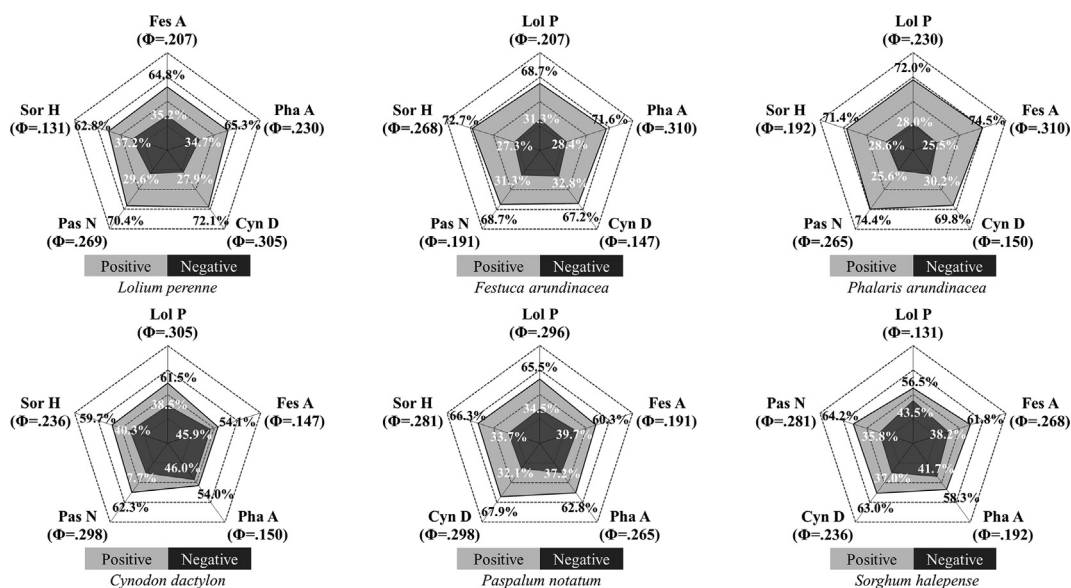


Fig. 3 Cross-reactivity between allergen extracts of selected species. Five extracts of allergens are represented on each pentagon. By visualizing each of these five extracts independently, it is assumed that absolutely all patients have a positive SPT for each extract indicated at the vertex of the pentagon. Similarly, visualizing each of these five extracts independently, the proportions of patients with positive and negative SPT to the pollen noted below the pentagon can be observed. All the proportions presented in the graph revealed a statistically significant association ($p < .05$) through a Chi-square test, and the strength of such association was measured by the Phi coefficient indicated in each vertex of the pentagon according to the allergen extract. Lol P, *Lolium perenne*; Fes A, *Festuca arundinacea*; Pha A, *Phalaris arundinacea*; Cyn D, *Cynodon dactylon*; Pas N, *Paspalum notatum*; Sor H, *Sorghum halepense*; SPT, skin Prick test; Φ , Phi coefficient

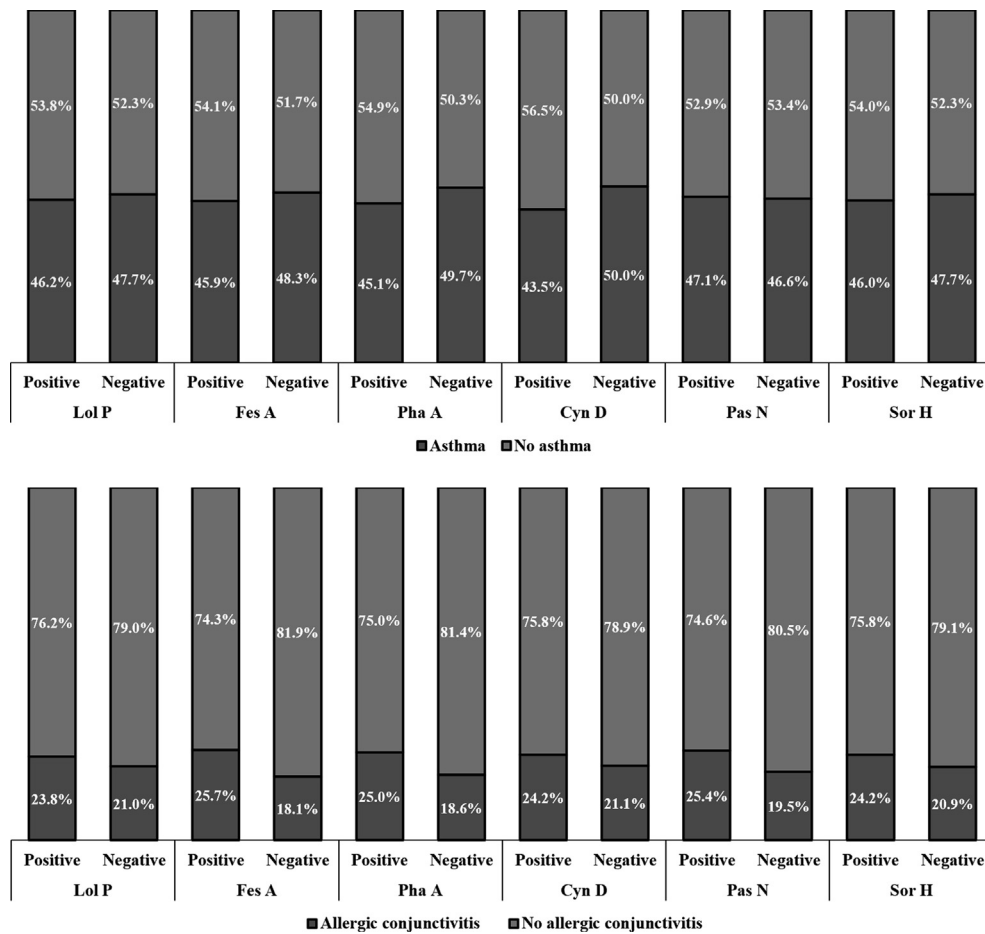


Fig. 4 Proportions of patients with asthma symptoms and allergic conjunctivitis by SPT response to allergen extracts of selected species. Proportions are expressed as percentages. Lol P, *Lolium perenne*; Fes A, *Festuca arundinacea*; Pha A, *Phalaris arundinacea*; Cyn D, *Cynodon dactylon*; Pas N, *Paspalum notatum*; Sor H, *Sorghum halepense*

When assessing specific extracts from each subfamily, sensitivity to *Phalaris arundinacea* was the most common (62.2%), followed closely by the other extracts of the *Pooideae* subfamily (*Festuca arundinacea*, and *Lolium perenne*). We also found lower reactivity to allergens from the *Chloridoideae* (*Cynodon dactylon*) and *Panicoideae* (*Paspalum notatum*, *Sorghum halepense*) sub-families when compared to the *Pooideae* subfamily, with a statistically significant difference in the size of papules size ($\chi^2(5) = 83.75$, $p < .001$). Such observation contrasts with findings from another subtropical region, where *Cynodon dactylon*, and *Paspalum notatum* exhibited greater sensitivity measured by SPT than *Lolium perenne*.¹⁵ In Europe, differences in level of reactivity have also been observed and appear to be related to the patient's country of origin. For instance, a lower degree of SPT response to *Cynodon dactylon* was found when compared to temperate grass

pollens extracts, such as *Lolium Perenne*.¹⁶ These observations further exemplify the regional differences seen in allergic sensitivity to different types of subtropical grass pollens.¹

In *Poaceae*, although species belonging to different sub-families are characterized by distinct allergen subsets, there is a considerable degree of cross-reactivity between many species, even between temperate and subtropical grasses. For example, in IgE cross-inhibition assays performed in patients with grass pollinosis in India, five locally abundant subtropical grasses: *Cynodon*, *Cenchrus*, *Imperata*, *Pennisetum*, and *Sorghum*, revealed asymmetric IgE cross-inhibition consistent with the subfamily of grass pollens.¹⁷ Albeit not a direct comparison, we found that patients with a positive SPT to a specific extract were more likely to present some degree of cross-reactivity to the remaining pollens when

compared to patients with negative SPT to the same specific extract. These findings might be the result of structurally-related proteins (e.g. profilins, polcalcins, lipid-transfer proteins) responsible for cross-reactivity among different allergenic sources.^{18,19} However, since our analyses revealed that such associations were weak, it can be concluded that regardless of SPT response to a certain extract, patients still need to be screened for all the remaining pollens. Further studies through IgE cross-inhibitions assays should be performed to determine the exact extent of the observed cross-reactivity.

The clinical implications of grass pollen allergy are mainly related to the triggering of allergic diseases in sensitized patients.⁴ Peaks in airborne grass pollen in late spring and early summer can give rise to exacerbations of allergic rhinitis, allergic conjunctivitis, and asthma.³ For instance, a study in Australia found the levels of airborne grass pollen to be a strong independent non-linear predictor of hospital admissions related to asthma.²⁰ In our study, even though roughly 40% of patients with seasonal allergic rhinitis also presented asthma, its proportion was slightly higher in patients with negative SPT in some grass species when evaluating each specific extract. However, the correlation between asthma and SPT response was not statistically significant. On the other hand, even though allergic conjunctivitis was less frequent than asthma in our sample, we found a statistically significant association between its presence and sensitization to *Festuca arundinacea*, *Phalaris arundinacea* and *Paspalum notatum* extracts, with frequencies of sensitization around 25%. In comparison, a study of the most common allergens in Northern Greece found that 58.4% of patients with allergic conjunctivitis presented a positive SPT to a grass mix.²¹

Finally, there are several limitations in our study that require mentioning. First, our results were restricted to the availability of allergens representing the vegetation of our region. Second, since an extract to *Paspalum quadrifarium* was not available, which is a true representative of the region, *Paspalum notatum* was used instead, leading to indirect comparisons despite its taxonomic

affinity. Moreover, cross-reactivity was assessed by SPT response, however, cross-inhibition IgE assays should be performed to formally establish cross-reactivity. Finally, since we did not screen for other allergen sensitizations besides pollen, airborne fungus and dust mites, we cannot guarantee that patients were monosensitized exclusively to pollen, and thus, we cannot explain if other antibodies take part in the cross-reactivity since antibodies to other allergens not included in this study may also cross-react to the allergens tested in the study if such antigens share similar epitopes. To the best of our knowledge, this is the first study to assess specific grass pollinosis among patients with seasonal allergic rhinitis from Bahía Blanca, Argentina.

CONCLUSION

Our results suggest a high frequency of sensitization to grass pollen extracts from the *Poaceae* family among patients with seasonal allergic rhinitis. Overall, sensitization to the *Pooideae* subfamily was the most common, where *Phalaris arundinacea* presented the highest frequency (62.2%). Positive reactions to allergens from the *Chloridoideae* and the *Panicoideae* subfamilies exhibited lower SPT response than allergens from the *Pooideae* subfamily. Even though the proportion of patients presenting with asthma (46.9%) was higher than those with conjunctivitis (22.6%), there was only a statistically significant association between sensitization to *Festuca arundinacea*, *Phalaris arundinacea*, and *Paspalum notatum*, with conjunctivitis.

Abbreviations

SPT: skin prick test; CI: confidence interval

DECLARATIONS

Ethics approval and consent to participate

This study was approved by the ethics committee Comité de ética e Investigación en Seres Humanos (CEISH) in accordance to the principles established by the declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interest

The authors declare no relevant conflicts of interest related to this work.

Author's contributions

Authors have made substantial contributions to conception and design, acquisition, analysis and interpretation of data, have been involved in drafting the manuscript or revising it critically for important intellectual content, and given final approval of the version to be published. GDR, LBB, VV participated in the recollection of data. EV performed the statistical analyses. GDR, EV, MF, ICO participated in the interpretation of analyses, figures, tables, and drafting of the manuscript. EV, MF collaborated in revising and improving the final version of the manuscript. All authors read and approved the final version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.waojou.2019.100062>.

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