

# Extensive Cross-reactivity Between *Salsola kali* and *Salsola imbricata*

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## ■ Abstract

**Background:** There are no studies on cross-reactivity between *Salsola kali* and *Salsola imbricata* pollens. The main goals of the present study were to compare the degree of the cross-reactivity between *S kali* and *S imbricata* and to compare the various allergenic components shared by *S kali* and *S imbricata*.

**Methods:** Serum samples were obtained from rhinitis patients with or without asthma living in Kuwait and presenting with a positive skin test result to *S kali*. SDS-PAGE/IgE Western blot and ELISA inhibition assay were performed.

**Results:** The study population comprised 37 patients. The most frequent IgE proteins against *S imbricata* weighed around 12, 15, 18, 37, and 50+55 kDa. 2D electrophoresis revealed a correlation between *S kali* and *S imbricata* at 40, 60, and 75 kDa, with similar isoelectric points. ELISA inhibition revealed an Ag50 value of 1.7 µg/mL for *S kali* and 500.5 µg/mL for *S imbricata* when the solid phase was *S kali* and an Ag50 value of 1.4 µg/mL for *S kali* and 3.0 µg/mL for *S imbricata* when the solid phase was *S imbricata*.

**Conclusions:** ELISA inhibition revealed strong cross-reactivity between *S kali* and *S imbricata*. This finding might be clinically relevant for the efficacy of allergen-specific immunotherapy. We report, for the first time, the allergenic profile of *S imbricata* and potentially allergenic proteins for *S kali* and *S imbricata*.

**Key words:** Allergens. Epitopes. IgE. Immunoblotting. Immunotherapy. Rhinitis. *Salsola*.

## ■ Resumen

**Introducción:** No existen estudios sobre la reactividad cruzada entre pólenes de *Salsola kali* y *Salsola imbricata*. El objetivo principal de este estudio es comparar el grado de reactividad cruzada entre *Salsola kali* y *Salsola imbricata*, y el objetivo secundario es comparar los diversos componentes alérgicos entre *Salsola kali* y *Salsola imbricata*.

**Métodos:** Se obtuvo suero de pacientes con rinitis con o sin asma, que vivieran en Kuwait y tuvieran un test positivo a *Salsola kali*. Se realizaron SDS PAGE/IgE Western blot, ELISA inhibición, a los sueros de pacientes kuwaitíes.

**Resultados:** Se incluyeron 37 pacientes kuwaitíes. Las proteínas que reaccionaron con más frecuencia contra *Salsola imbricata* se encontraron alrededor de 12, 15, 18, 37 y 50+55 kDa. La electroforesis 2D mostró una correlación entre *Salsola kali* y *Salsola imbricata* a 40, 60, 75 kDa con puntos isoeléctricos similares. El estudio de ELISA inhibición mostró un Ag50 de 1,7 µg/mL para *Salsola kali* y 500,5 µg/mL para *Salsola imbricata* cuando la fase sólida era *Salsola kali*, y un Ag50 de 1,4 µg/mL para *Salsola kali* y 3,0 µg/mL para *Salsola imbricata* cuando la fase sólida era *Salsola imbricata*.

**Conclusión:** *Salsola kali* y *Salsola imbricata* presentan una fuerte reactividad cruzada como se observa en el ELISA inhibición y esto podría ser clínicamente relevante para la eficacia de la inmunoterapia específica contra alérgenos. Hemos descrito por primera vez el perfil alérgico para *Salsola imbricata* y los posibles alérgenos comunes entre *Salsola kali* y *Salsola imbricata*.

**Palabras clave:** Alérgenos. Epitopos. IgE. Inmunoblotting. Inmunoterapia. Rinitis. *Salsola*.

## Introduction

Most patients with respiratory allergies in the State of Kuwait are sensitized to *Salsola pollens* [1], which are a predominant source of sensitization in the Gulf region [2,3] and Iran [4], as *Salsola* is prone to grow in salty soils in areas with low rainfall [5,6]. *Salsola* is also found in other dry countries such as the USA, Australia, and Spain [7-9]. Kuwait, a country of about 18 000 km<sup>2</sup> in the northwestern Arabian Gulf, has an arid climate. Natural vegetation is sparse and includes many species from the Chenopodium/Amaranthaceae family. *Salsola imbricata* grows widely in most parts of Kuwait and flowers in the summer, especially in September and October, when most pollen-sensitized patients become symptomatic. *Salsola kali*, however, has not been reported in Kuwait [10], although pollens can travel over considerable distances [11]. In the case of *Chenopodium* and *Amaranthus* species, the most prevalent plants in Kuwait are *Chenopodium murale* and *Amaranthus lividus* [10].

Studies in Kuwait have shown that *Chenopodium* pollens are the dominant type and are at their highest level during September and October [1].

Immunotherapy is an effective treatment for allergic rhinitis and bronchial asthma [12,13] and has proven efficacious with *S kali* [14]. Our allergy center has been using commercially available *S kali* instead of *S imbricata* pollen extracts for both skin testing and specific immunotherapy. About 70% of pollen-sensitized patients seen at our clinic react to *S kali* extract. However, the rationale for using an extract from a different, although closely related pollen for skin testing and immunotherapy has been questioned.

The main aims of this study were to compare cross-reactivity between *S kali* and *S imbricata* pollen extracts and to compare allergen components between *S kali* and *S imbricata* pollens.

Characterization of allergens of *Salsola* species has mainly been carried out with *S kali* [7,15]. *S kali* is the major allergen, with a large number of isoforms. Since *S imbricata* allergens have not been characterized, no studies have shown the allergenic profiles of both *S kali* and *S imbricata* pollens.

## Material and Methods

### Study Population

The inclusion criteria were residence in Kuwait, treatment at the Al-Rashed Allergy Center, allergic rhinitis with or without asthma, and a positive skin test result to *S kali*. The diagnosis of allergic rhinitis was based on a compatible clinical history and symptoms affecting the nasal mucosa and turbinates, and asthma was confirmed in all cases as FEV<sub>1</sub> (% predicted) <80% in at least 1 measurement, with improvement of ≥12% after inhalation of a bronchodilator (salbutamol). Sensitization to *S kali* was confirmed using skin prick tests (Diater). Written consent was obtained from all patients, and the study was approved by the Ethics Research Committee, Ministry of Health, Kuwait.

### Skin Testing

All patients were tested with a battery of aeroallergens that included *Dermatophagoides pteronyssimus*, cat and

dog dander, *Alternaria*, *Aspergillus*, *Cladosporium*, *Phoenix dactylifera*, *Olea europea*, 5-grass mix, *Bermuda*, *Plantago*, *Amaranthus*, *Artemisa*, *Chenopodium album*, and *S kali*. Skin prick tests were performed by a specially trained nurse on the volar surface of the forearms using skin prick test reagents (Diater) and metal lancets. The tests included a positive control (histamine solution) and a negative control (normal saline). The results were read after 15 minutes, and a wheal reaction of ≥3 mm was considered positive.

### Specific IgE

Levels of specific IgE to *S kali* were measured using the ImmunoCAP specific IgE assay (Thermo Fisher Scientific). Values ≥0.35 kU<sub>A</sub>/L were considered positive.

### *S imbricata* and *S kali* Pollens

Pollen from locally grown *S imbricata* was obtained by careful vacuuming and drying of plants. Pollen from *S kali*, *C album*, and *Amaranthus retroflexus* and peanut extract were purchased from Allergon. Proteins from all pollens and peanut were obtained in phosphate-buffered saline (PBS) (1.37 mM NaCl, 14.7 mM KH<sub>2</sub>PO<sub>4</sub>, 78.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 26.8 mM KCl) at pH 7.4. The homogenate was shaken magnetically for 30 minutes at 5±3°C. The aqueous extract was obtained by filtering the soluble fraction through an AP 20 glass fiber filter (Merck Millipore) comprising a glass fiber prefilter and an 0.8-mm membrane (AP membrane 2009000, Merck Millipore). It was then dialyzed against deionized water with membranes with a molecular cut-off of 3500 Da (Visking, Iberlabo) for 16 hours at 5±3°C before being stabilized by freeze drying.

### SDS PAGE/IgE Western Blot

Proteins from *S kali*, *S imbricata*, *C album*, *A retroflexus*, and peanut extracts were analyzed using SDS-PAGE according to Laemmli [16] in 15% polyacrylamide gels under reducing conditions. Proteins were visualized using Coomassie Brilliant Blue R-250 staining and electrophoretically transferred to a polyvinylidene difluoride membrane (BioRad). IgE antibody binding to allergens was analyzed by Western blot using the patients' sera and antihuman IgE peroxidase conjugate (Southern Biotech). Chemiluminescence detection reagents (Western Lightning Plus-ECL, Perkin Elmer) were added. IgE binding bands were identified using the BioRad Diversity database program.

2D electrophoresis was performed using BioRad reagents and equipment. For the first dimension, 7-cm IPG strips (pH 3-10). These were hydrated at 50 V and 20° for 12 hours with 150 µL of rehydration solution (8M urea, 50 mM DTT, 2% [wt/vol] CHAPS, and 0.2% [wt/vol] Byo-Lite pH 3-10) containing 100-300 µg of each protein sample. Isoelectric focusing was conducted at 20°C as follows: S1, rapid voltage at 250 V for 15 minutes; S2, rapid voltage at 4000 V for 2 hours; S3, rapid voltage from 4000 to 10 000 V; and S4, rapid voltage at 500 V for 20 minutes. The limit voltage was 50 µA/gel strip. After focusing, the gel strips were equilibrated with equilibration solution I (0.375 M Tris-HCl at pH 8.8, 6 M urea, 20% [vol/vol] glycerol and 2% [wt/vol] DTT) and equilibration solution II (0.375 M Tris-HCl at pH 8.8, 6 M urea, 20% [vol/

vol] glycerol, 2% [wt/vol] SDS and 2.5% [wt/vol] IAA) by gentle shaking for 10 minutes. The IPG strip was embedded onto an SDS-PAGE gel (12.5% separating gel). Gels were run at a constant current of 120 V for 20 minutes. The current was raised to 200 V per gel until complete.

### ELISA Inhibition Assays

According to the level of cross-reactivity between *S kali* and *S imbricata* pollen extract in patients' sera, inhibition experiments were performed using the pollen extracts from *S kali* and *S imbricata*. ELISA inhibition was carried out

using a modified protocol of Ceska and Lunkwist [17]. Briefly, 12.5 µg/well of *S kali* pollen extract in carbonate buffer (15 mM Na<sub>2</sub>CO<sub>3</sub> and 35 mM NaHCO<sub>3</sub>, pH 9.6) was incubated at 4°C overnight in a 96-well microtiter plate (Nunc MaxiSorp). Each well was blocked for 1 hour at room temperature with 100 µL of 1% BSA in PBS-Tween 0.05%, followed by incubation for 2 hours with pool sera and the specific amounts of each pollen extract preincubated at room temperature. The wells were incubated as follows: 30 minutes at room temperature with a 1:1000 dilution of antihuman IgE monoclonal E-27 IgG (Operon) in 1% BSA in PBS-Tween 0.05%; 30 minutes at room temperature with 1:500 dilutions of biotinylated antimouse

Table. Results of Specific IgE Testing

#	Age	Sex	Disease	sIgE Sal k, kU <sub>A</sub> /L	sIgE Sal k 1, kU <sub>A</sub> /L	Country of Origin	Other Pollen <sup>a</sup>	Other
1	22	F	Rhinitis and asthma	5.62	11.9	Kuwait	Ber	Cat
2	60	M	Rhinitis and asthma	12.9	70	Egypt		Cat
3	45	F	Rhinitis and asthma	9.81	27	Kuwait	Amr, Ber	
4	24	M	Rhinitis	1.44	9.48	Kuwait		
5	31	M	Rhinitis and asthma	15.9	39.5	Kuwait	Art, Ber, Che, Pln	Cat, Alt, date
6	23	M	Rhinitis and asthma	>100	>100	Kuwait	Art, Ber, Che	Date
7	29	F	Rhinitis and asthma	3.4	0.08	Kuwait		
8	52	F	Rhinitis and asthma	0.49	0.02	Kuwait	Che	
9	33	F	Rhinitis and asthma	18.3	18.9	Kuwait	Amr, Che	
10	30	F	Rhinitis and asthma	3.19	10.9	Syria	Che	Nuts
11	50	F	Rhinitis and asthma	14.5	44.4	Bangladesh	Ber, Che	
12	44	M	Rhinitis and asthma	2.16	13.5	Kuwait		
13	33	M	Rhinitis and asthma	4.23	6.59	Egypt		
14	16	M	Rhinitis and asthma	3.03	40.9	Kuwait	Ber	
15	21	M	Rhinitis	2.69	26.2	Kuwait		Cat
16	58	M	Rhinitis and asthma	0.27	1.17	Egypt	Che	
17	50	F	Rhinitis and asthma	41.4	>100	Bangladesh		
18	48	M	Rhinitis and asthma	4.04	0.27	Bangladesh		
19	58	F	Rhinitis and asthma	1.19	3.38	Kuwait	Ber, Che	
20	30	M	Rhinitis and asthma	3.98	11.5	Kuwait	Che	Alt
21	56	M	Rhinitis and asthma	11.1	45	Pakistan		Cat
22	34	F	Rhinitis and asthma	8.5	29.8	Kuwait		
23	22	F	Rhinitis	3.89	20.3	Kuwait		Dp
24	48	F	Rhinitis and asthma	2.78	8.23	Egypt		
25	31	F	Rhinitis and asthma	1.87	4.13	Kuwait	Ber	Date
26	36	M	Rhinitis	5.58	7.02	Kuwait		
27	47	M	Rhinitis and asthma	10.08	33.9	Kuwait	Ber, Che	
28	46	M	Rhinitis	25.04	79.5	Egypt		
29	49	M	Rhinitis and asthma	2.44	2.63	Kuwait		Cat
30	42	F	Rhinitis and asthma	8.85	32.3	Kuwait	Art, Che	Cat
31	60	M	Rhinitis and asthma	4.47	9.03	Egypt	Amr, Ber	
32	26	F	Rhinitis	13	44.1	Kuwait	Che	
33	55	M	Rhinitis and asthma	4.38	33	Kuwait	Ber, Che	
34	49	F	Rhinitis and asthma	10.08	27.7	Kuwait	Che	
35	20	M	Rhinitis	1.39	4.95	Kuwait		
36	33	M	Rhinitis and asthma	20.07	77.2	Egypt	Che	Cat
37	19	M	Rhinitis and asthma	3.25	16.08	Kuwait	Ber	Cat, Dp

Abbreviations: Alt, *Alternaria*; Amr, *Amaranthus*; Art, *Artemisia*; Ber, *Bermuda*; Che, *Chenopodium*; Dp, *Dermatophagoides pteronyssinus*; Pln, *Plantago*.

<sup>a</sup>Pollen species other than *Salsola* to which the patient had a positive skin test result.

IgG (Sigma Aldrich Química) in 1% BSA in PBS-Tween 0.05%; and 30 minutes with streptavidin peroxidase (Sigma Aldrich Química) at 1:250 in 1% BSA in PBS-Tween 0.05% and then with 50 µl of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).

## Results

The study population included 37 patients (21 males and 16 females) with a mean age of 37.4 years (range, 15-60). Six patients presented with allergic rhinitis alone, and 31 presented with allergic rhinitis and asthma. All patients had lived in Kuwait for longer than 5 years.

All 37 patients had a positive skin test result to *S kali*, with a wheal diameter of 4 mm to 15 mm (mean wheal size, 7.88 mm).

Specific IgE levels were measured in all patients for both *S kali* and recombinant Sal k 1, and all patients tested positive (Table).

### Protein Profiles of Pollens

Proteins from *S kali* and *S imbricata*, were separated using SDS-PAGE under reducing conditions (Figures 1 and 2).

### IgE-Binding Profiles of Pollen Extracts

Reactivity of sera to *S kali* and *S imbricata* pollens is shown in Figures 1 and 2. Several IgE binding components were revealed, with weights ranging from 10 to 75 kDa. As it was not possible to determine any bands for *S imbricata* in the case of patients 10, 12, 22, and 26, these were excluded from the final results.

The IgE components that were most frequently reactive to *S kali* pollen extract were a 43-kDa protein that was compatible

with Sal k 1 and several proteins of 22 kDa and 70 kDa. The most frequent IgE components reactive to *S imbricata* pollen extract were several proteins at around 12, 15, 18, 37, and 50+55 kDa.

Western blot performed with *C album*, *A retroflexus*, and peanut did not show any consistent pattern of coincidence with the reactive proteins for *S kali* and *S imbricata* extracts (Figure 2).

The proteins from *S imbricata* and *S kali* were separated on a series of 2D gels (Figure 3A) run in parallel with a pH gradient of 4.5 to 8.5 and subsequently transferred to a PVDF membrane for incubation with a pool of sera from allergic patients. Several proteins from *S imbricata* (Figure 3B) with a molecular size of approximately 15 to 100 kDa were recognized by IgE, including the following: 100 kDa (pI, 4.7-5.1), 75 kDa (pI, 5.5-5.7), 60 kDa (pI, 4.7-5.3), 55 kDa (pI, 5.6-7.0), 50 kDa (pI, 4.5), 40 kDa (pI, 5.7-7.0), 30 kDa (pI, 5.1-5.9), 20 kDa (pI, 4.8-5.6), and 15 kDa (pI, 4.5-8.5). IgE recognized proteins from *S kali* at 100 kDa (pI, 5.2-5.7), 75 kDa (pI, 5.2-8.0), 60 kDa (pI, 4.5-5.0), 50 kDa (pI, 5.4-5.7), 40 kDa (pI, 5.1-6.8), 30 kDa (pI, 5.4-5.9), 23 kDa (pI, 4.7), and 18 kDa (pI, 4.8).

### Allergenic Potency and Cross-reactivity

To determine the cross-reactivity of sera to *Salsola* species, we determined Ag50 values for each pollen extract using ELISA inhibition (Figure 4). The Ag50 value was 1.7 µg/mL for *S kali* extract and, theoretically, 500.5 µg/mL for *S imbricata* when the pollen extract coated to the solid phase was *S kali*; however, when the pollen extract coated to the solid phase was *S imbricata*, the Ag50 value of *S kali* pollen extract was 1.4 µg/mL and the Ag50 value of *S imbricata* was 3.0 µg/mL.

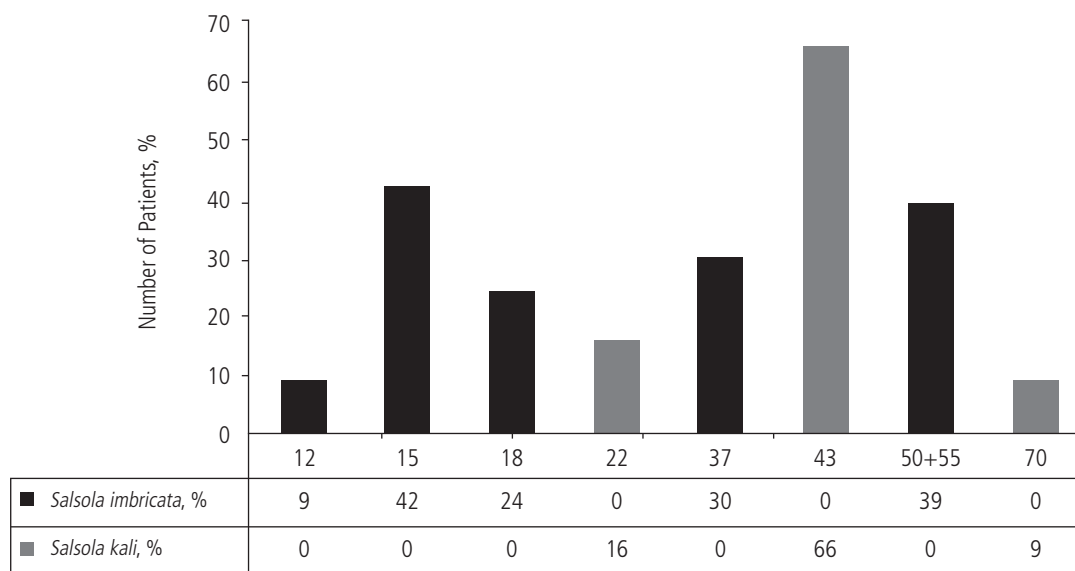


Figure 1. Frequency of IgE reaction with SDS-PAGE-separated allergens of *Salsola kali* and *Salsola imbricata* pollen extracts.



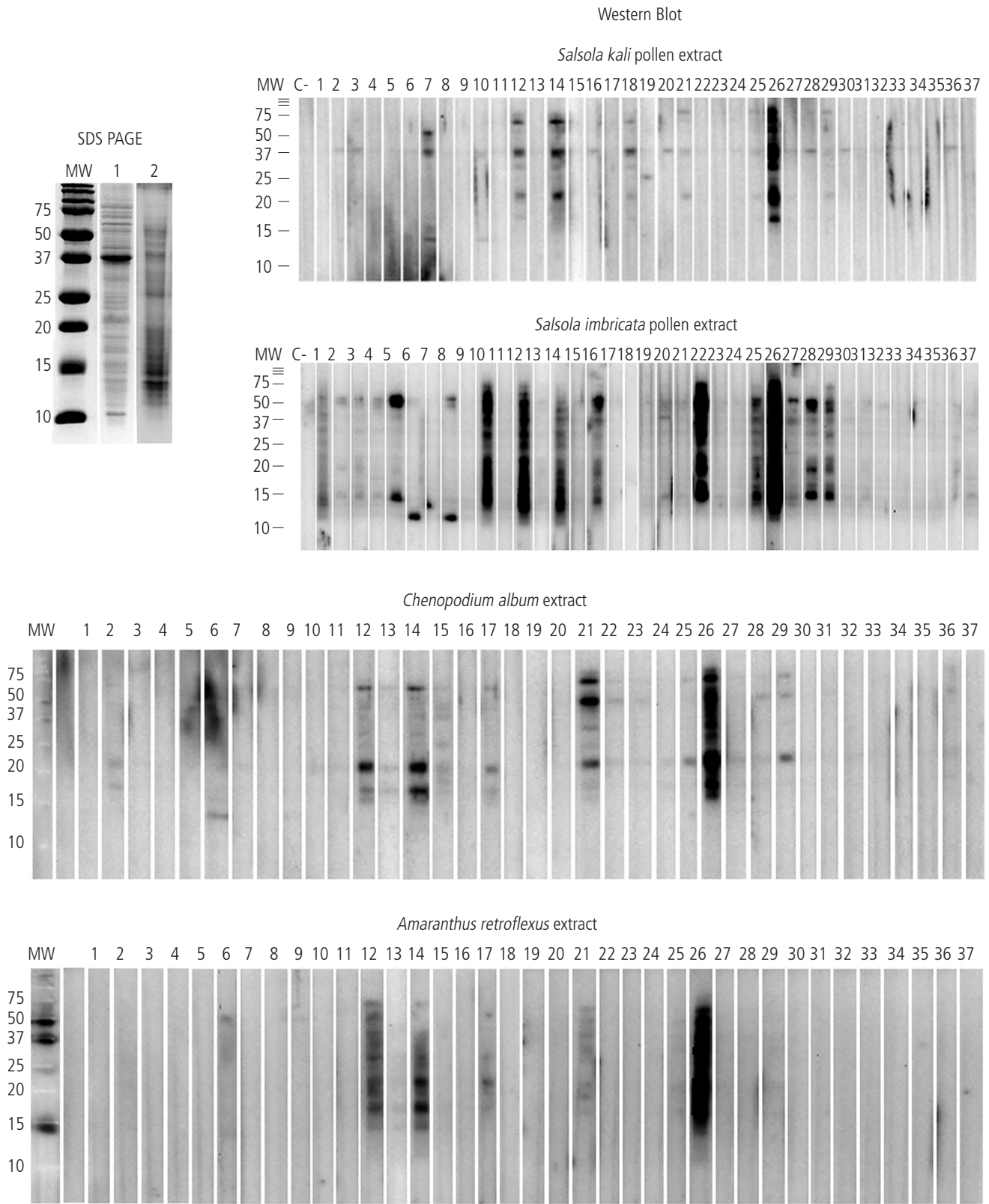
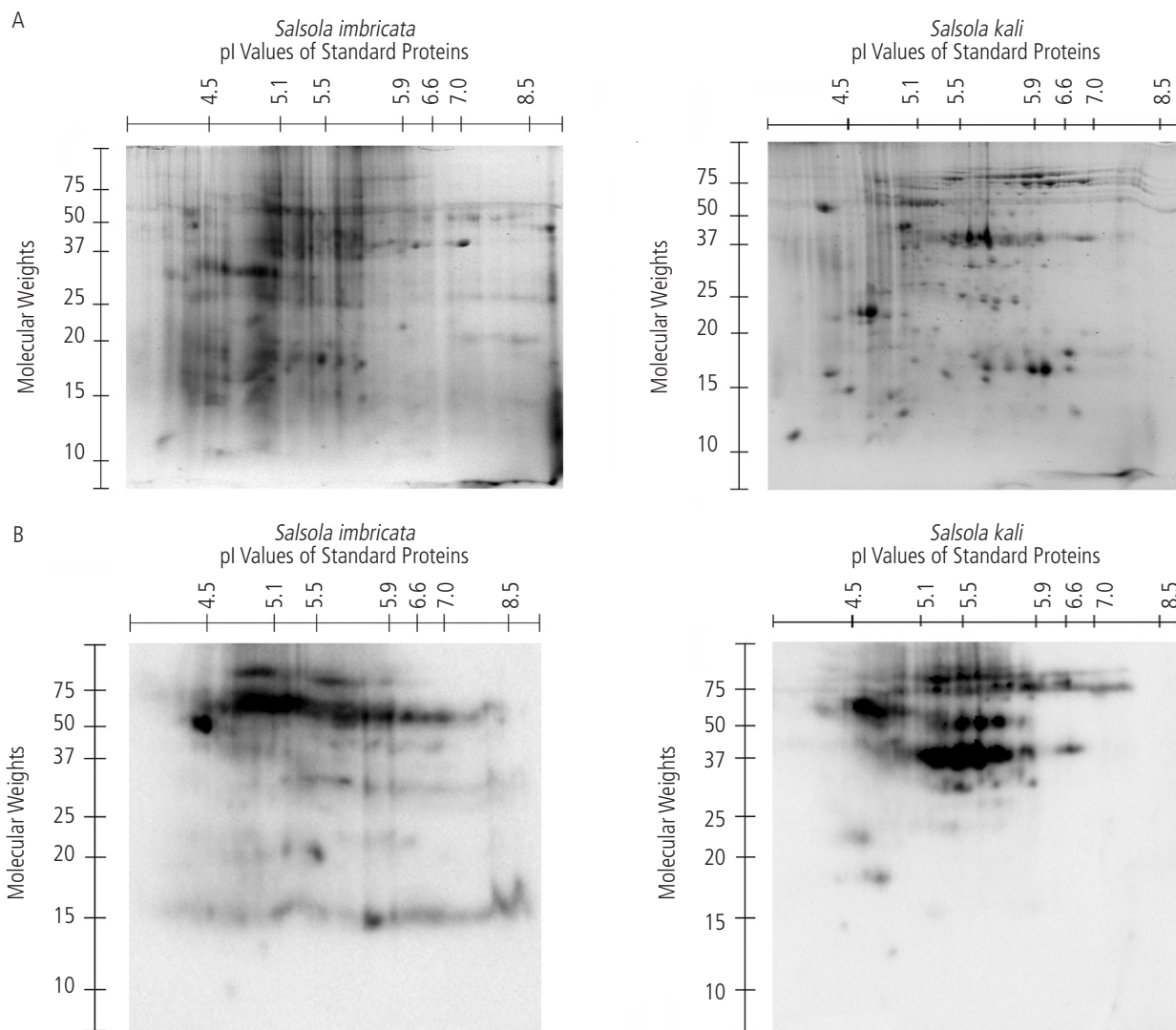


Figure 2. SDS-PAGE performed with *Salsola kali* (lane 1) and *Salsola imbricata* (lane 2). Western Blot performed with *Salsola kali*, *Salsola imbricata*, *Chenopodium album*, *Amaranthus retroflexus*, and patients' serum.



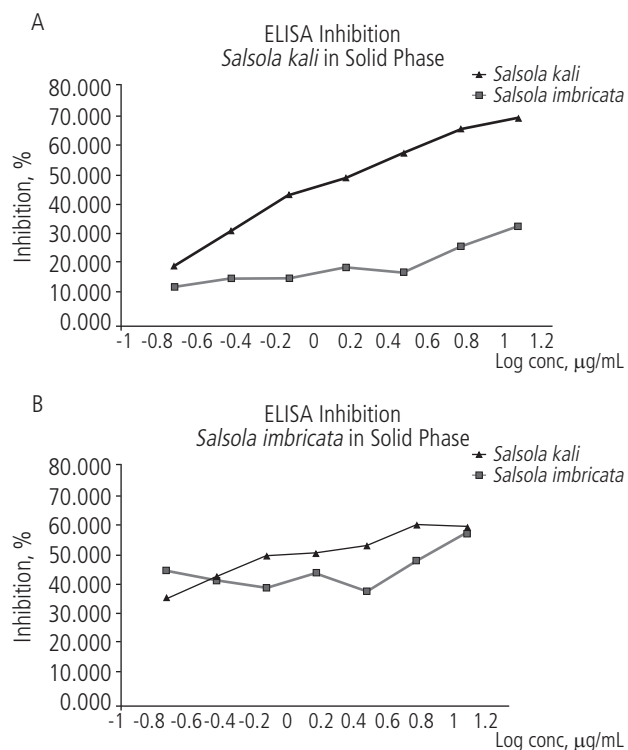
**Figure 3.** SDS-PAGE (A) and western blot (B) 2D of allergenic extract of *Salsola kali* vs *Salsola imbricata*, with a pool of sera from patients sensitized to *Salsola imbricata*.

## Discussion

The allergenic profile of *S imbricata* pollen has not been studied; therefore, major and minor *S imbricata* allergens have not been described or isolated. However, cross-reactivity between *Salsola* and other species of *Chenopodium* is well established [7]. The binding observed for *S imbricata* at 50+55 kDa and 37 kDa has not been previously reported, and the allergen is not predominant in any of the most common pollens of Kuwait such as *Chenopodium* (10-17kDa), *Bermuda* (9-32 kDa), and *Amaranthus* (14,18 kDa) [1,18]. In order to find a common source of sensitization that could explain possible cross-reactivity patterns for *S kali* and *S imbricata*, Western blot was performed with *C album* and *A retroflexus*; however, no clear correlations with any of the major binding

components obtained were observed. Western blot was performed with peanut owing to previously described cross-reactivity with *S kali* proteins [19]. Binding with peanut has not been described in other predominant pollens from the Gulf region, such as *Prosopis juliflora* (18 kDa) [3] Furthermore, *Phoenix dactylifera* presents with allergens at 14, 40, and 90 kDa. Binding at 12, 15, and 18 kDa could be related to profilins, although the nature of these bands should be characterized.

Increased prevalence of Sal k 1 in patients sensitized to foods and other pollens has been reported [20]. However, when the bindings obtained for *S kali* and *S imbricata* were correlated patient-by-patient, we found no clear relationship with cosensitization to other pollens or foods that could clarify the possible cross-reactivity with Sal k 1 or any of the main



**Figure 4.** ELISA inhibition of allergenic extract of *Salsola kali* and *Salsola imbricata* with *Salsola kali* as solid phase. B, *Salsola kali* vs *Salsola imbricata* with *Salsola imbricata* as solid phase.

bands detected for *S imbricata*. Similarly, no clear correlation was observed between Sal k 1 and the main bands detected for *S imbricata*. This finding was somewhat unexpected, as all patients had positive skin test results and specific IgE against *S kali* and several bands were clearly recognized for *S kali*. Moreover, we found no correlation with the country of origin. Although *S kali* is not present in Kuwait [10], we are aware of the possibility that *S kali* pollen might travel long distances from nearby regions and hence be a source of sensitization for the study patients. Since patients living in Kuwait do not seem to clearly recognize the Sal k 1 counterpart in *S imbricata*, it is unlikely that inhibition of Sal k 1 would give clear results in the inhibition assay.

We detected 3 areas of common reactivity in 2D Western blot. The first was at 40 kDa (pI, 5.7-7.0) for *S imbricata* and 40 kDa (pI, 5.1-6.8) for *S kali*, both of which findings correlate with those previously described for Sal k 1 [7] [18], although the bands for *S imbricata* were weaker, suggesting that cross-reactivity may be only partially explained by a common Sal k 1 protein. The second area was found at 75 kDa (pI, 5.5-5.7) for *S imbricata* and 75 kDa (pI, 5.2-8.0) for *S kali*; this area might be related to Sal k 3. The third area was at 60 kDa (pI, 4.7-5.3) for *S imbricata* and 60 kDa (pI, 4.5-5.0) for *S kali*, although these findings require further characterization.

The main goal of the present study was to compare cross-reactivity between *S kali* and *S imbricata* owing to the clinical implications of a theoretically decreased response to *S kali*

immunotherapy in a country where *S kali* is not present. Despite the incomplete concordance between *S kali* and *S imbricata* IgE binding profiles on Western blot, ELISA inhibition presented similar potency for inhibition when *S imbricata* was in the solid phase and a clear preference for *S kali* when *S kali* was in the solid phase. Consequently, binding was stronger for the predominantly European *S kali* than for the local *S imbricata*. This may be because our patients are exposed to a number of other highly cross-reacting pollens (Chenopods and Amaranthaceae).

Aerobiology studies in Kuwait have shown a recurring pattern: the total atmospheric pollen count increases in summer, with a lesser peak in March/April and a significantly more pronounced peak in October/November. These periods also correspond to increased symptoms in the study patients. Pollens in March/April comprise mainly grass pollen but also Chenopod/Amaranthaceae pollens; in October/November they consist mainly of Chenopod/Amaranthaceae pollens. *C album* and *C murale*, together with several *Amaranthus* species (*Amaranthus hybridus*, *Amaranthus lividus*), are fairly common. However, nearer to inhabited areas, *S imbricata* is abundant on disturbed ground and along roadsides, where it flowers throughout summer, although more markedly in October/November, before it fruits.

The absence of greater similarity between the protein patterns in the SDS-PAGE analysis of the pollens of *S kali* and *S imbricata*, together with the strong inhibition by *S kali* allergens in ELISA, suggests that other Chenopod/Amaranthaceae pollens may be involved in the sensitization of the study patients, who are routinely skin tested (and often undergo specific IgE testing) with pollen extracts of *S kali*, *C album*, and *A retroflexus*. *S kali* is almost always the most reactive.

A recent study of adherence to *S kali* immunotherapy in Kuwait [21] indirectly suggests that subcutaneous immunotherapy with *S kali* is effective in the study population, who are exposed to *S imbricata* pollen but not to *S kali* pollen. The results of ELISA inhibition suggest that immunotherapy with *S kali* extracts could be even more effective than immunotherapy with local *S imbricata* extracts, although further studies are needed to confirm this observation.

The limitations of the study include differences in the methods used to collect *S kali* and *S imbricata* pollens, which might have affected the results, even though the protein concentrations for immunoblot and ELISA inhibition were equal. The collection process could have specifically affected the protein profiles of *Salsola* owing to the presence of immature or already germinated pollen at the time of collection. Methacholine challenge testing was not performed in patients presenting with allergic rhinitis and no clinical evidence of bronchial asthma in order to check for evidence of airway hyperresponsiveness. Further studies are needed to confirm these findings.

In conclusion, *S kali* and *S imbricata* present with a strong pattern of cross-reactivity, as shown in ELISA inhibition. We report the first allergenic profile for *S imbricata* with protein bands at 12, 15, 18, 27, and 50+50 kDa on immunoblotting in patients where sensitization to *S imbricata* is predominant. We describe several possible common allergenic proteins

for *S kali* and *S imbricata* at 40, 60, and 75 kDa with similar isoelectric points.

### Funding

The authors declare that no funding was received for the present study.

### Conflicts of Interest

Dr. Pineda reports being employed by Diater Laboratories, a pharmaceutical company specializing in the production of allergenic extracts for in vivo diagnosis and treatment of allergies. The remaining authors declare that they have no conflicts of interest.

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