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ORIGINAL ARTICLE

Allergy to *Salsola Kali* in a *Salsola Incanescens*-rich Area: Role of Extensive Cross Allergenicity

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ABSTRACT

Background: Pollens from the Salsola spp. are an important source of respiratory allergy in tropical countries. Our aim was to characterize the IgE binding proteins of *S. incanescens* pollen extract and study its cross-reactivity with *S. kali* pollen allergens.

Methods: Prick tests with *S. kali* and *S. incanescens* pollen extracts were performed on eight respiratory allergy patients from Mashhad, Northeast Iran. The antigenic profiles and IgE-binding patterns of *S. kali* and *S. incanescens* pollen extracts were compared by SDS-PAGE and Western blotting, using individual sera from the salsola pollen-sensitive patients. Cross-reactivity of proteins in the two weeds was assessed by IgE- immunoblotting inhibition.

Results: *S. kali* and *S. incanescens* pollen extracts showed similar IgE-binding profiles in Western blotting. The IgE binding components of 39, 45, 66 and 85 kDa were detected in both pollen extracts. Furthermore, inhibition of the immunoblots revealed extensive inhibition of IgE binding to proteins and a close relationship between these two weeds allergens.

Conclusions: *S. incanescens* pollen is a potent allergen source with several IgE binding components that shows a close allergenic relationship with *S. kali*. Our results suggest that in *S. incanescens*-rich areas, *S. kali* pollen extracts could be used as a diagnostic reagent for allergic patients to *S. incanescens* pollen.

KEY WORDS

cross allergenisity, *Salsola incanescens*, *Salsola Kali*

INTRODUCTION

Pollens from the Chenopodiaceae family have been reported as an important source of pollinosis in the Western United States, European countries and Asia.[1] Furthermore, Chenopodiaceae sensitization is a severe problem in semidesertic countries such as Saudi Arabia, Iran and Kuwait.[2-4] The genus Salsola is one of the well- known genera of the Chenopodiaceae family and its species, especially *S. kali* (formerly *S. pestifer*) and *S. incanescens*, are common throughout arid and semiarid regions of Iran.[4,5] It would be important to point out that *S. incanescens* is more abundant than *S. kali* in Mashhad, Northeast Iran. In addition, the inhalation of Salsola spp. pollen is the most important cause of allergic respiratory symptoms in Iran. It occupies the first place in the prevalence of sensitizations observed in our outpatient clinic and affects 75% of patients with clinical sensitivity to pollens.[6,7] Shafiee *et al*. reported two allergenic proteins with molecular weight of 39.0 and 42.0 kDa in the Salsola pestifer pollen.[4] Sal k 1, a 43 kDa allergen in *S. kali* has been recently described.[1,5] This allergen was characterized using serum samples from patients residing in Spain. Beside this, sensitization produced by *S. incanescens* is not documented and the commercial *S. incanescens* pollen extract is not available commercially. In this study, we compared the IgE cross-reactivity between *Salsola kali* and *S. incanescens* using *in vivo* and *in vitro* assessments.

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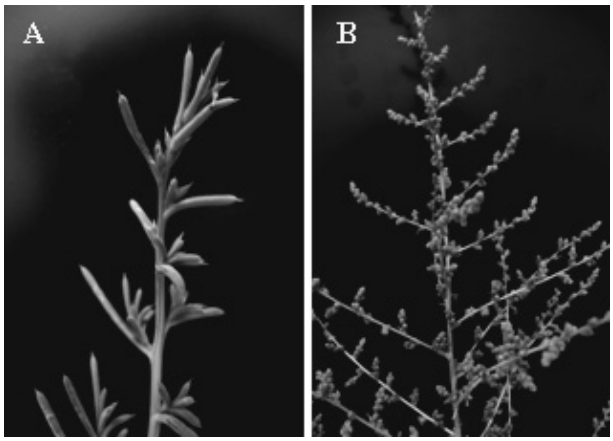

Fig. 1 **A**. *Salsola kali*; **B**. *Salsola incanescens*

METHODS

PREPARATION OF EXTRACT

The Salsola kali pollen (purchased from Greer Laboratories, USA) had a purity of more than 95%. Polleniferous materials were collected from *S. incanescens* flowers throughout disturbed or unoccupied sites in Mashhad, a city in northeast of Iran with a population of more than 2,300,000 (Census 2006), during June–September. It grows on any type of well-drained uncompacted soil with a sunny exposure. The morphology and anatomical features of *S. incanescens* and *S. kali* showed different characteristics (Fig.1).

Collection and processing of pollen materials were done carefully by trained pollen collectors. Pollen grains were separated by passing the dried materials through different sieves (100, 200 and 300 meshes) successively. The final fine powder was subjected to a purity check for pollen content using a microscope. Pollen materials with more than 95% pollen and less than 5% floral parts of the same plant were taken for antigen extraction.

Pollen materials were defatted using repeated changes of diethyl ether. Two grams of pollen were extracted in 10 ml phosphate-buffered saline (PBS) 0.01 M (pH 7.4) by continuous stirring for 18 hours at 4℃. The supernatant was separated by centrifugation at 14,000 g for 30 minutes, filtered and the supernatant collected. The extract was centrifuged at 16,000 g, filtered through a 0.22 μm membrane under sterile condition and dialyzed against 10 mM phosphate buffer. The extract was then freeze-dried. The protein content of the extract was measured by Bradford's method.[8]

PATIENT POPULATION AND SKIN TEST

A total of 8 patients who presented to the Immunology Research Center of Bu-Ali Research Institute with seasonal rhinitis, without asthma, participated in the study. The patients were all positive by Skin prick test (SPT) with *S. kali* and *S. incanescens* extracts. The Human Ethics Committee of the institute approved the study protocol with informed written consent from each patient.

Skin prick tests were performed by an experienced nurse under a physician's supervision. In this test, *S. kali* and *S. incanescens* pollen extracts were put on the patients' inner forearms and irritation of the epidermis was caused by prick method. The result was observed after 15 minutes. Next, the mean diameter of wheal reaction in every patient was measured and compared with negative (Glycerol saline) and positive (Histamine, 10 mg/ml) controls. Patients with a wheal diameter >3 mm were considered positive compared with negative and positive controls and were asked to donate a serum sample. Serum samples of patients were stored at −20℃ before use.

SDS-PAGE AND IgE-IMMUNOBLOTTING

The *S. kali* and *S. incanescens* extracts were fractionated by means of SDS-PAGE in accordance with the method of Laemmli [9] on Bio-Rad Miniprotean II System gels (12.5% polyacrylamide) and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes, as described elsewhere.[10] In brief, after washing and blocking, membranes were incubated with a serum pool or individual sera from patients with *S. kali* and *S. incanescens* allergy or with control sera (1 : 5 dilution). Biotinylated anti-human IgE (KPL, USA) (1 : 1000 v/v in 1% BSA) was added to the blotted membrane strips and incubated for 2 hours at room temperature. The unbound antibodies were removed from blots by washing with PBS and followed by incubation with 1 : 20000 v/v in BSA1% HRP- linked streptavidin (Bio-Rad, USA) for 2 hours at room temperature. The bound enzymatic activity of horseradish peroxidase was detected by enhanced chemiluminescence (Attoglow, UK) and documented with G: box imaging system (Syngene, UK).

IgE-IMMUNOBLOTTING INHIBITION

To study the cross-reactivity between *S. kali* and *S. incanescens* pollen allergens, the IgE-immunoblot inhibition experiment was performed. SDS-PAGE resolved *S. kali* pollen proteins were transferred to PVDF membrane. After blocking, membrane strips were kept for 3 hours at room temperature with a mix of 100 μl of pooled sera (1 : 5 v/v) which were preincubated with equal volume of *S. incanescens* protein extract (70 μg/ml, as inhibitor) and BSA (as negative control) for 3 hours. Membrane strips were washed three times with PBS incubated with Biotin-labeled anti-human IgE (KPL, USA) (1 : 1000 v/v in 1% BSA) for 2 hours followed by 1 : 20,000 HRP-linked streptavidin (Bio-Rad) incubation for 1 hour at room temperature. Blots were developed using chemiluminescence reagents (Attoglow). The procedure was repeated by incubating the pooled sera with extracts of *S. kali*.

Table 1 Clinical characteristics, skin reactivity and total IgE values

S. no.	Age (years)/sex	Clinical history †	Skin test grading ‡	
			S. kali	*S. incanescens*
Patients				
1.	28/M	AR	+++	+++
2.	20/F	AR, RC	+++	+++
3.	23/F	AR	++	++
4.	29/M	AR	++	++
5.	27/F	AR, RC	+++	++
6.	26/F	AR	++	++
7.	36/F	AR, RC	+++	+++
8.	25/M	AR	++	++
Controls				
9.	36/F	AR, RC	—	—
10.	23/M	—	—	—
11.	28/M	—	—	—
12.	38/F	AR, RC	—	—

† AR, Allergic rhinitis; RC, rhiniconjectivits.

‡ ++, equivalent to positive control (histamine diphosphate 10 mg/ml); +++, more than positive control.

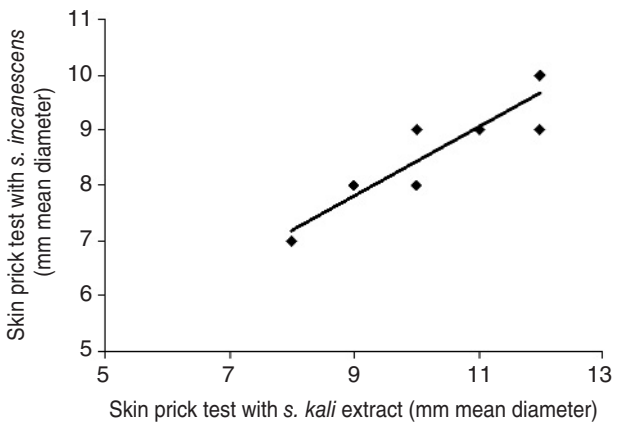

Fig. 2 Linear Correlation between *S. kali* and *S. incanescens* weal diameter (mm) of prick test ($r = 0.91$, $p < 0.002$).

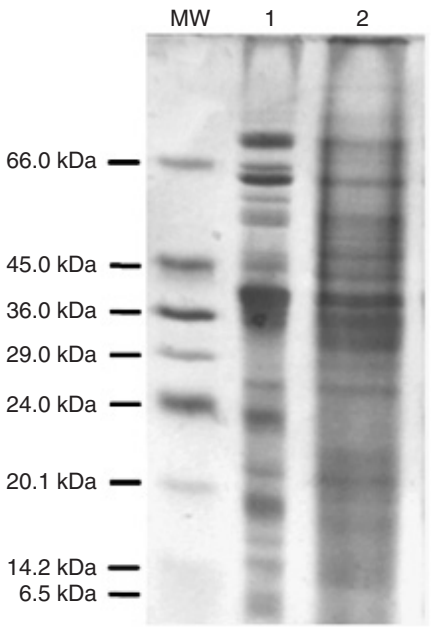

Fig. 3 SDS-PAGE of whole *S. kali* and *S. incanescens* extracts. Lane MW, low molecular weight (sigma); lane 1, whole *S. kali* extract; lane 2, whole *S. incanescens* extract.

RESULTS

SKIN REACTIVITY

Eight patients, 5 women and 3 men (mean age, 26.75±4.71 years; age range 20–36 years), presenting to the Immunology Research Center of Avicenna Research Institute with seasonal rhinitis, without asthma, were all positive using SPT with *S. kali* and *S. incanescens* extracts (Table 1). A serum pool of 4 non-allergic subjects was used as a negative control. We compared mean weal diameter (mm) of skin prick test with *S. kali* extract (x axis, mm mean diameter) with the results of skin prick test with *S. incanescens* extract (y axis, mm mean diameter) (Fig.2). Linear regression analysis showed a strong correlation between the two skin prick tests ($r = 0.91$. $p < 0.002$). The Human Ethics Committee of the institute approved the study protocol with informed written consent from each patient.

PROTEIN AND ALLERGENIC PROFILE

The protein composition of both extracts was analyzed by silver staining (Fig.3). The SDS-PAGE separation of the pollen extracts showed similar protein profiles in the two weeds. There were several resolved protein bands in the *S. kali* and *S. incanescens*

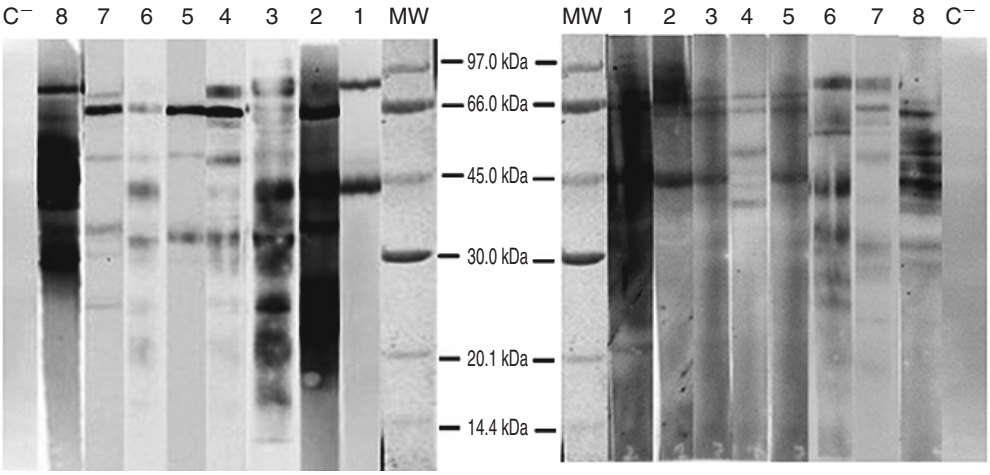

Fig. 4 IgE Immunoblot of *S. kali* (left) and *S. incanescens* (right) pollen extracts using eight hypersensitivity patients' sera. Lane MW, low molecular weight (Amersham, UK); lanes 1−8, probed with individual patients' sera; lane c⁻, negative control.

Table 2 Frequency of protein reactivity in *S. kali* and *S. incanescens* extracts

Protein molecular weight (kDa)	Frequency of reactivity (%)	
	S. kali	*S. incanescens*
25	37.5	12.5
39	62.5	37.5
45	75	75
66	75	75
85	62.5	37.5

extracts with molecular weights in the range of approximately 18 to 85 kDa. There were more bands and greater intensity of bands with a molecular mass of more than 39 kDa in *S. kali*. The allergenic profile of pollen extracts was studied by immunoblot (Fig.4). The IgE-binding profile of *S. kali* pollen extracts was mainly composed of a major reactive band of 66 kDa, and four allergenic components of 25, 39, 45 and 85 kDa (Table 2). In *S. incanescens* the IgE-binding profile of pollen extract was mainly composed of two major reactive bands of 45 and 66 kDa and two minor allergenic components of 39 and 85 kDa. To confirm the specificity of the detection and obtain further information on proteins involved in the cross-reactivity, immunoblot-inhibition tests were carried out. For immunoblot inhibition studies, 100 µl of pooled sera (1 : 5 v/v) from the patients with allergy to *S. kali* and *S. incanescens* pollen was incubated for 3 hours at room temperature with 70 µg/ml inhibitor or BSA (negative control). The preadsorbed serum was then used for immunoblot experiments.

Like *S. kali* extract, 45 and 66 kDa components of *S. incanescens* extract were strongly recognized by the IgE antibodies of the patients with *S. kali* allergy (Fig.5). No band was detected when a negative control serum pool was assayed (not shown). Complete inhibition of IgE binding to 39, 45, 66 and 85 kDa components of *S. kali* extract occurred when the sera were preincubated with *S. incanescens* extract at 70 µg/ml. Meanwhile the one band at 25 kDa was partially inhibited. There was no inhibition of IgE binding when the sera were incubated with 0.1 µg/ml of BSA. IgE binding to *S. incanescens* extract was strongly inhibited by *S. kali* extract using pooled sera.

DISCUSSION

This is the first study to evaluate cross-reactivity between *S. kali* and *S. incanescens*. These members of Chenopodiaceae family are major weeds throughout arid and semi-arid areas of Iran.

It is of interest to point out that *S. incanescens* was found several times more than *S. kali*; unfortunately, there is no study on *S. incanescens* allergenicity in Iran or other countries. On the other hand, *S. kali* has been recognized as one of the most common causes of allergic rhinitis in our region and other desert and semi-desert areas.[6]

Our results indicate that the mean wheal diameter (mm) of skin prick tests with *S. kali* extract showed strong correlation with the results obtained by *S. incanescens* extract (r = 0.91. p < 0.002). However, wheal diameter of skin prick tests with *S. kali* extract was larger than *S. incanescens*. This difference may be because we used the home made *S. incanescens* extract for prick test which has a purity which was less than the commercial extract of *S. kali*. Therefore, it could be anticipated that, in the case of using commercial extract, more skin prick test reactivity would be found to occur. Although, some of the *S. kali* pollen allergens have been characterized,[1,4,5] there is

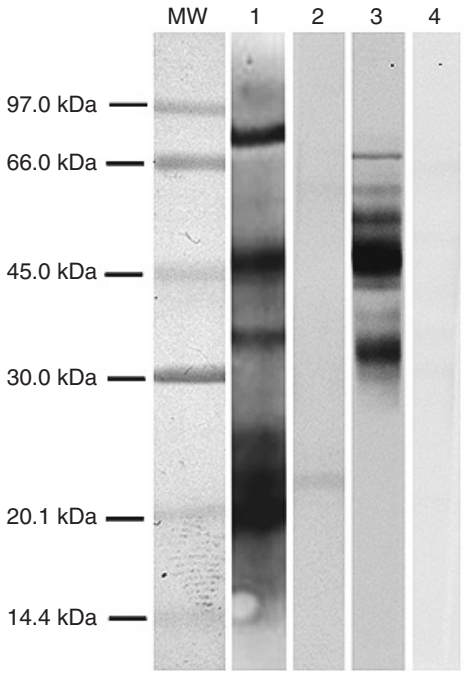

Fig. 5 IgE immunoblot inhibition with *S. incanescens* pollen extract. Lane MW, low molecular weight (Sigma); lanes 1, *S. kali* protein strip probed with pooled sera from patients number 1, 2, 3, 7 with reactivity to 25, 39, 45 and 85 kDa components, without inhibitor; lane 3, *S. kali* protein strip probed with pooled sera from patients number 4, 5, 6 and 8 with reactivity to 66, 45 and 39 kDa components, without inhibitor; lanes 2 and 4, *S. kali* protein strips incubated with two serum pools (from patients number 1, 2, 3, 7 and 4, 5, 6, 8, respectively) containing 70 μg/ml of *S. incanescens* pollen extract as inhibitor.

limited information regarding allergenic components of *S. kali* pollen and their immunological relationship to components present in other allergen sources.

To study the allergen profile of *S. kali* and *S. incanescens* pollens, we used sera from a group of eight *S. kali* and *S. incanescens* pollen-sensitive patients living in the city of Mashhad and exposed them to high loads of these weed pollens in the summer. Our results showed that IgE in the sera of patients with allergy to *S. kali* recognize protein components with the similar molecular weight in both Salsola spp. pollen extracts. In the pollen extract of *S. incanescens*, the 39, 45, 66 and 85 kDa components exhibited the most immunological similarities with the pollen allergen components of *S. kali*. The inhibition studies also indicated that these proteins had extensive cross-reactivity. Our results clearly indicated extensive cross-reactivity between *S. kali* and *S. incanescens* which belong to the same genera. This is supported by previous studies, in which the repeatability of the amino acid sequences reaches 80–90% in species of the same genus.[11] Cross-reactivity among pollens from a similar genus and/or different genera has been demonstrated earlier.[12] Moreover, cross-reactivity were observed between the allergens of grass pollen within one genus, also between the genera, and the antigens of birch and ash tree pollen,[11,13] within the *Fagaceae* family.[11,14] Some cross-reactive proteins were genetically engineered and could be used in immunotherapy.[15]

In previous studies [1,3,16] significant cross-reactivity were described between Salsola and Chenopodium spp. These results were also inconsistent with our immunoblotting inhibition experiments, which showed extensive cross reactivity of *S. kali* with *Chenopodium album* and some degree with *Amaranthus rtroflexus*, *Kochia scoparia*, *Artemisia douglasiana* (data not shown). It is noteworthy that the patients in our study were most often sensitized to at least three allergenic pollen extract from different members of the Amaranthaceae/Chenopodiaceae family. Multi-allergen sensitization could be due to long-term exposures with allergens belonging to close reservoirs,[17] cross-reactivity of allergen and interactions of genetic and environmental factors. In conclusion, *S. incanescens* pollen is a potent allergen source with several IgE binding components. *S. incanescens* showed a close allergenic relationship with *S. kali*. Regarding the extensive cross-reactivity between these two weeds and also abundant *S. incanescens* in Mashhad, *S. incanescens* pollen could be involved in sensitization of *S. kali* pollen allergic patients. As in our outpatient clinic the pollen extract of *S. incanescens* was not available, it could be possible that prick test with *S. kali* pollen extract shows allergic reaction despite the fact that *S. incanescens* pollen was involved in the sensitization. Moreover, the observations suggest that 39, 45, 66 and 85 kDa proteins could be used as diagnostic and therapeutic reagent for patients allergic to *S. kali* and *S. incanescens*.

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