

# Identification of 2 New Allergens of *Phoenix dactylifera* Using an Immunoproteomics Approach

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## ■ Abstract

The date palm (*Phoenix dactylifera*) has a wide geographical distribution (Middle East, Mediterranean, central Africa, western Asia, Australia, and North America). Pho d 2, the major allergen of date palm pollen was recently identified as a profilin, yet little is known about the nature of the other pollen allergens from this tree.

The objective of this study was to characterize clinically significant allergens other than profilins from *P dactylifera* pollen using immunoproteomics.

In order to reveal the proteins causing the allergy, we used serum from a patient monosensitized to date palm pollen extract who experienced asthma and rhinoconjunctivitis during the palm tree pollen season.

The results revealed 2 novel immunoglobulin E-binding proteins not related to the cross-reactive allergen profilin. Individualized allergens of *P dactylifera* that cause specific date palm pollen sensitization must be defined to determine the real prevalence of sensitization to this species.

**Key words:** *Phoenix dactylifera* allergens. Palm tree. Allergy.  $\beta$ -Galactosidase precursor.  $\alpha$ -1,4-Glucan protein synthase.

## ■ Resumen

La palmera datilera es un árbol de amplia distribución geográfica que se puede encontrar en zonas de Oriente Medio, en los Países Mediterráneos, África Central, Asia Occidental y Norte América. Recientemente ha sido definido el alérgeno mayor del polen de palmera (Pho d 2), incluyéndose en el grupo de las profilinas de origen vegetal. No obstante y hasta el momento no se conoce con exactitud la naturaleza y/o función de otros alérgenos presentes en el polen de esta especie.

El objetivo de este estudio ha sido la caracterización de alérgenos no relacionados con Pho d 2 que puedan tener un significado clínico en la alergia al polen de palmera, utilizando la proteómica como propuesta para la identificación de nuevos alérgenos. Para ello se utilizó el suero de un paciente monosensibilizado al polen de palmera que exhibía rinitis y asma en la época de polinización de este árbol.

Los resultados demostraron la existencia de dos nuevas proteínas fijadoras de IgE no relacionadas con la profilina. Estos datos indican que es necesario definir de una forma individualizada, cada uno de los alérgenos específicos implicados en la sensibilización al polen de palmera, para poder calcular los datos de sensibilización a esta especie que presenta la población así como sus implicaciones clínicas.

**Palabras clave:** *Phoenix dactylifera* alérgenos. Palmera. Alergia. Precursor de  $\beta$ -galactosidasa.  $\alpha$ -1,4-glucan protein sintasa.

## Introduction

The date palm (*Phoenix dactylifera*) belongs to the Aracaceae family and is distributed throughout the Middle East, Mediterranean countries, central Africa, western Asia, Australia, and North America [1].

Palm pollen grains are predominant aeroallergens in tropical and subtropical regions, and sensitization to this pollen has been shown to represent an important cause of pollinosis in these regions [2]. Exotic ornamental plants, such as the date palm, are increasingly common in public places, and it is known that its pollen is a potent allergen [3]. Only the major allergen of the date palm (Pho d 2) has been fully characterized. It has been described as a profilin [4], although very little is known about the nature of the other allergens of *P dactylifera* pollen and their possible role in the development of clinical symptoms. In this report, we study 2 new date palm pollen allergens identified by 2D-immunoblotting and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

## Case Description

A 40-year-old woman experienced asthma and rhinoconjunctivitis during the palm tree pollen season. We used her serum to identify the protein components causing the allergy. Skin prick testing and specific immunoglobulin (Ig) E (ImmunoCAP, Phadia, Uppsala, Sweden) using a standard allergen panel (*Dermatophagoides pteronyssinus*, *Alternaria alternata*, *Aspergillus fumigatus*, *Penicillium notatum*, *Cladosporium herbarum*, cat and dog epithelia, and pollens from *Phleum pratense*, *Olea europaea*, *Parietaria*

*judaica*, *Salsola kali*, *Chenopodium album*, *Mercurialis annua*, *Platanus orientalis*, *Betula verrucosa*, *Pinus radiata*, *Artemisia vulgaris*, *Cupressus arizonica*, *Plantago lanceolata*, and *P dactylifera*) revealed positive results only with *P dactylifera* pollen extract.

The allergen profile, analyzed using ImmunoCAP ISAC (Phadia), did not show positive results, and specific IgE to profilins, including Pho d 2, was undetectable. These results suggested that the patient was monosensitized to date palm pollen.

In order to identify the proteins involved in the allergic reaction, the date palm pollen extract (BIAL, Aristegui, Bilbao, Spain) was analyzed using 2D-electrophoresis, which was performed following standard methods. Briefly, samples were loaded onto immobilized pH gradient (IPG) strips (pH 3-10) (ReadyStrips, BioRad, California, USA) for the first-dimension run. The strips were focused using the PROTEAN IF cell (BioRad) according to the manufacturer's instructions. The focused IPG strips were then incubated, first in equilibration buffer and next in the same buffer containing iodoacetamide. The equilibrated strips were applied to the surface of vertical 10% sodium dodecyl sulfate polyacrylamide gels and the proteins were separated in the second dimension using the MiniPROTEAN® III separation unit (BioRad). After protein separation, gels were either fixed and stained for total protein using Coomassie Brilliant Blue R-250 or transferred to a polyvinylidene fluoride membrane [5].

The membranes were blocked and incubated overnight with the patient's serum at 4°C with shaking. After incubation for 1 hour with mouse antihuman IgE (Fc) horseradish peroxidase conjugate (SouthernBiotech, Birmingham, Alabama, USA) and subsequent washings, the membranes were treated with

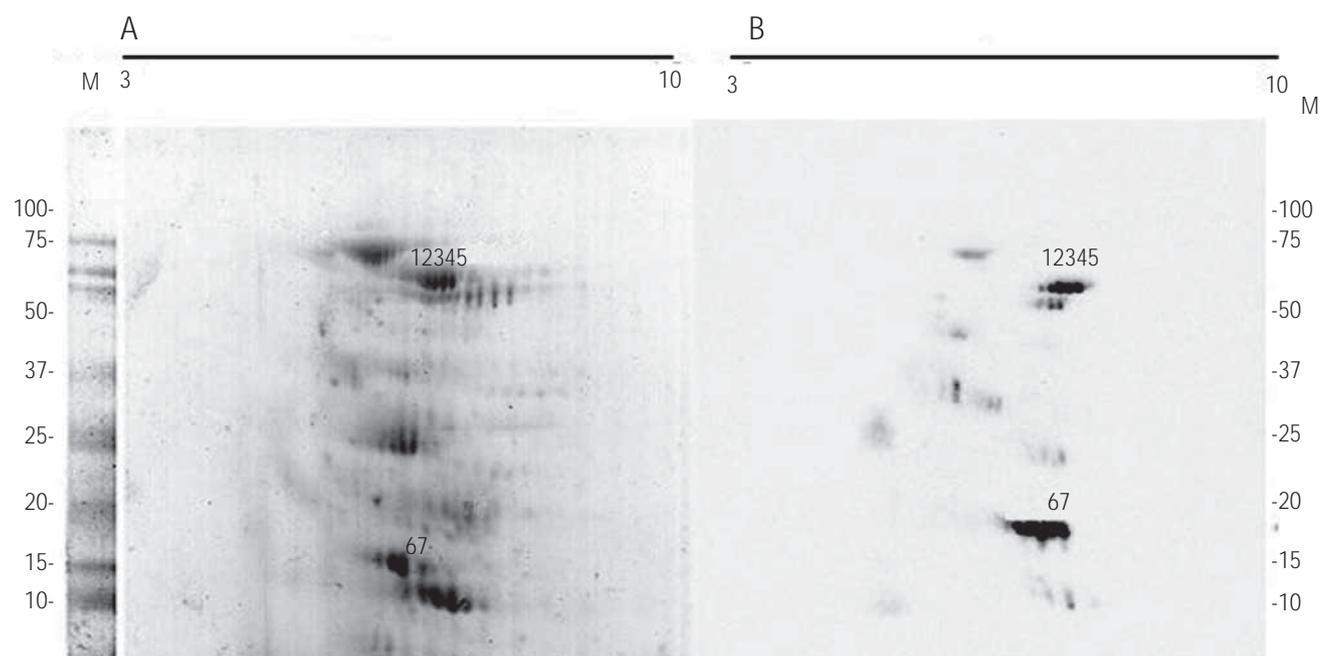


Figure. Spectrum of proteins in date palm pollen separated by 2D electrophoresis. A, visualized by Coomassie Blue staining. B, recognized by IgE antibodies of the monosensitized patient. The numbering refers to the Table. M indicates molecular weight marker (kDa); pI, isoelectric point.

the ECL chemiluminescent substrate (Amersham ECL Plus Western Blotting Detection System, GE Healthcare UK Ltd, Buckinghamshire, UK) [6]. The results were recorded using the ChemiDoc XRS System (BioRad).

Peptide mass fingerprinting using MALDI-TOF-MS and/or de novo sequencing by MS/MS analysis was used to analyze the IgE binding spots revealed by 2D-immunoblotting (Proteomic Unit, Centro Nacional de Investigaciones Cardiovasculares, Instituto de Salud Carlos III, Madrid, Spain). Trypsin digestion was carried out according to Schevchenko et al [7]. Peptide mass fingerprints were obtained by MALDI-TOF-MS [8] and/or de novo sequencing by MS/MS analysis according to Gautam et al [9].

MALDI-TOF-MS and MS/MS data were used to interrogate protein databases (NCBIInr, National Center for Biotechnology Information, Bethesda, USA; and SwissProt, Swiss Institute of Bioinformatics, Switzerland) using the MASCOT search program (Matrix Science, London, UK) [10].

Figure 1A shows the proteomic profile of date palm pollen. Several components with molecular weights ranging from 9 to 100 kDa and pIs ranging from 5 to 8 were revealed. Figure 1B shows the 2D-immunoblotting profile of date palm pollen extract revealed from the patient's serum. Five IgE binding components with a molecular weight of 90 kDa and a pI of 6 to 7.5, and 2 with molecular weights of 20 kDa and pIs of 5.5 to 7 were detected.

The Table shows protein identification by MS analysis of the IgE immunoreactive proteins. The results showed that spots 1 to 5 revealed sequences that match 1% with a  $\beta$ -galactosidase precursor (lactase 10) from *Oryza sativa*. This protein (accession number P85412), with a pI of 6.2 and a molecular weight of 90.7 kDa, is involved in the hydrolysis of terminal nonreducing  $\beta$ -D-galactose residues in  $\beta$ -D-galactosides. Spots 6 and 7 match 17% and 11%, respectively, with the type IIIa membrane protein cp-wap13 ( $\alpha$ -1,4-glucan protein synthase) from *Vigna unguiculata*, which is involved in the synthesis of cellulose. The pI and molecular weight for this protein (accession number P85413) were 6.2 and 40.8 kDa, respectively.

## Discussion

The date palm has an extensive geographical distribution [1] and is widely used in food and gardening. Allergies caused by this species have been described in the Middle East [2], India [11], and Mediterranean countries [12]. In Spain, sensitization prevalence values to the date palm tree range from 5.6% to 29.4% [12,13].

Several allergenic components of date palm pollen have been described, but only 1 has been exhaustively characterized and identified as a profilin [4]. Kwaasi et al [14] reported 6 allergen components with molecular weights of 12, 14, 28-30, 37-40, 57, and 65-67 kDa, binding 60%-93% of atopic sera, and they were named Pho d I to Pho d VI. Not one of these allergens has been characterized in detail and, as far as we know, no specific sensitization markers define palm tree pollen allergy.

In recent years, proteomics has proven to be an excellent approach to identifying novel allergens [15]. This technique enabled

Table. Protein Identification of the Immunoglobulin E Immunoreactive Proteins of Date Palm Pollen

Spot ID	Calculated pI/MW	Protein Identity	Matched Peptide sequence	Sequence Coverage %	MASCOT Score	Expected Value	NCBI Accession Number
1	6.23/90.75	Putative $\beta$ -galactosidase 10 precursor	EIQNAGLYAILR	1	133	2.8E-07	gi/75116245
2	6.23/90.75	Putative $\beta$ -galactosidase 10 precursor	EIQNAGLYAILR	1	120	5.6E-06	gi/75116245
3	6.23/90.75	Putative $\beta$ -galactosidase 10 precursor	EIQNAGLYAILR	1	115	1.8E-05	gi/75116245
4	6.23/90.75	Putative $\beta$ -galactosidase 10 precursor	EIQNAGLYAILR	1	128	8.9E-07	gi/75116245
5	6.23/90.75	Putative $\beta$ -galactosidase 10 precursor	EIQNAGLYAILR TIKVPGEFDYELYNRNDIN YVDAVLTIPK	1	121	4.5E-06	gi/75116245
6	6.24/40.08	Type IIIa membrane protein cp-wap13	VICDHLGLGVKTGLPYIWHKASNP FVNLK	17	455	1.8E-39	gi/2218152
7	6.24/40.08	Type IIIa membrane protein cp-wap13	TIKVPGEFDYELYNRNDIN VICDHLGLGVKTGLPYIWHK	11	166	1.4E-10	gi/2218152

Abbreviations: MW, molecular weight; NCBI, National Center for Biotechnology Information.

us to identify 2 novel date palm pollen allergens that match 1% with lactase 10 from *Oryza sativa*, and 17% with the membrane  $\alpha$ -1,4-glucan protein synthase from *Vigna unguiculata*.

Although the matching sequence percentage was very low in the case of lactase 10, identification of a homologous sequence from a database search guarantees that the 2 proteins have similar structures and often provides preliminary functional insights, even if the underlying alignment is poor. Comparison of MALDI-TOF/MS-MS results with protein-databases using statistical applications reveals full coincidences between peptides from 2 different digested proteins and includes them in the same protein-related group [16]. Nevertheless, the allergen detected and other allergenic proteins belonging to this family should be compared with caution. Only 2 previous reports have demonstrated the relationship between lactase and IgE mediated hypersensitivity [17,18].

No other allergens with  $\alpha$ -1,4-glucan protein synthase activity have been described to date.

Considering the high prevalence of date palm sensitization reported in Spain [12] and the definition of the *P dactylifera* major allergen as a profilin [4], a protein family widely involved in cross-reactions, caution should be exercised when associating prevalence values with allergenic species. It seems more likely that the values for date palm pollen sensitization in the Spanish population presented above [12] could be associated with individualized cross-reactive allergens such as profilins that could justify the high prevalence of sensitization in the general population.

Individualized allergens of *P dactylifera* that cause specific date palm pollen sensitization must be defined in order to calculate the real prevalence of sensitization to this species, and to establish better and more accurate diagnoses.

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