

RESEARCH ARTICLE

Proteome analysis of maize pollen for allergy-relevant components

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Over the last few decades, the cultivation of maize (*Zea mays*) has strongly increased in Central Europe. We therefore decided to study the allergen composition and the allergenic potency of its pollen in comparison with pollen from timothy grass (*Phleum pratense*), a typical representative of the native grasses. We found that 65% of the sera reactive to timothy pollen also bound to maize pollen proteins. By using 2-DE immunoblotting, followed by incubation with mAbs directed against known allergens or protein sequencing, those IgE-reactive components were further classified. Although novel, maize-specific pollen allergens could not be found, the presence of crossreacting allergens belonging to groups 1 and 13 (Zea m 1 and 13), both having high IgE prevalence, as well as the presence of the less important group 3 and 12 allergens was found. The structural variability of Zea m 1 and Zea m 13 was determined by sequencing clones isolated from a maize pollen cDNA library. This revealed sequence identities of 72 and 70%, respectively, to the corresponding Phl p 1 and Phl p 13 allergens of timothy grass pollen. IgE-crossreactivity was further studied using immunoblot inhibition tests. Here, timothy pollen extract completely blocked IgE binding to maize, whereas maize pollen extract blocked IgE reactivity to only some timothy pollen allergens.

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1 Introduction

Cultivation of *Zea mays* (maize, corn), a tropical grass from Mexico belonging to the Panicoideae subfamily, has rapidly increased in Europe. In Germany alone, maize tilling has increased three-fold over the last 20 years, now amounting to 1.7 million tons *per* year. Since pollen allergy affects about 10% of the population in industrialized countries, and numbers are still increasing, the determination of the allergenic potential of cultivated maize and its genetically engineered variants are of public interest [1, 2].

Allergies are immunological disorders, characterized by immune responses directed against harmless environmental substances, such as airborne grass pollen. Production of IgE antibodies in the allergic individual leads to allergic symptoms, ranging from rhinitis to bronchial asthma. In the last 20 years, many efforts have thus been made to characterize allergen-inducing molecules [3]. Grass pollen contains about 1000 different components, but only some are IgE reactive and can cause allergic reactions. Pollen allergens are classified according to their biochemical structure and immunological reactivity, leading to the identification of ten different allergen groups [4]. Table 1 presents an overview of timothy grass pollen allergens and their immunological and biochemical characteristics. Those allergens are also present in other members of the Pooideae subfamily that show a comparable proteome due to their close phylogenetic relationship [5, 6].

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Table 1. Immunological and biochemical characterization of the timothy grass pollen allergen groups (according to Behrendt and Becker [7] with modifications)

Allergen	IgE prevalence (%)	Molecular mass (kDa)	Function
Phl p 1	95	35	β-Expansin
Phl p 2	50	11	
Phl p 3	50	11	
Phl p 4	70	50	
Phl p 5a	90	38	RNase
Phl p 5b	90	32	
Phl p 6	60	13	
Phl p 7	7	9	Ca ²⁺ -binding protein
Phl p 11	40	20	Trypsin inhibitor
Phl p 12	10	14	Profilin
Phl p 13	60	60	Polygalacturonase

For diagnosis and specific immunotherapy (hyposensitization therapy), the extracts of allergy eliciting pollen are commonly used. However, in order to improve diagnosis and treatment, known allergens should be applied individually instead (component-resolved diagnosis), to determine a patient-specific reaction pattern. In the subsequent therapy, only those individual components that were previously identified as IgE reactive may then be applied to form well-defined therapeutics [8, 9]. Thus, the precise knowledge of allergenicity of individual pollen proteins is crucial. Maize pollen proteins and their putative function as aeroallergens have not been studied in detail yet. Our aim was to determine whether maize contains novel allergenic components and to which extent maize pollen may increase the allergenic burden. Since no maize pollen allergic individuals were available to us, we used sera of grass pollen allergic patients to determine and characterize putative IgE-reactive components in maize pollen. Additionally, we tested sera of individuals highly exposed to maize pollen for the presence of IgE antibodies directed against pollen proteins.

2 Materials and methods

2.1 Pollen extracts

One gram of *Phleum pratense* or maize pollen (Artu Biologics, Lelystad, Netherlands) was incubated in 50 mL of 0.1 M NH₄HCO₃ buffer (pH 8.0) for 30 min. The soluble fraction was isolated by centrifugation at 17 000 × g for 30 min and dialyzed against double-distilled water overnight. The extract was lyophilized and stored at 4°C until use.

2.2 Sera and mAb

Sera were drawn from individuals suffering from grass pollinosis with CAP classes >3 (>3.5 kU IgE/L) with their consent (kindly provided by Dr. Frank Eberhardt, Medical Clinic,

Borstel). Additionally, sera were drawn from four volunteers, who have been exposed daily to maize pollen for a few years and who assumed to have obtained a maize pollen allergy during greenhouse work. Skin prick tests of exposed individuals Ex 3 and 4 revealed a positive reaction to maize and timothy pollen extract. Sera from healthy blood donors served as controls.

A panel of nine mAbs directed against defined allergen grass groups had been raised against timothy grass pollen extract or a mixture of grass pollen species of temperate climate grasses in our laboratory. Bo1, Bo9, and BG6 are directed against group 5 allergens [6, 10], IG12, HB7, Bo14 recognize group 1 [11, 12], Bo12 group 6 [13], and AF6 and EG9 the group 13 allergens [14]. The mAbs 3C4 and 5H1 recognize group 4 allergens [15, 16] and were kindly provided by Professor Helmut Fiebig (Allergopharma, Reinbek, Germany).

2.3 Western blotting

SDS-PAGE was performed as described by Laemmli [17] on homogenous gels (*T* = 15%, *C* = 2.6%) under reducing conditions. Samples were loaded at concentrations of 5 µg/cm for timothy pollen and 10 µg/cm for maize pollen onto the gels. Determinations of molecular mass were achieved by use of the low range SDS-PAGE standards (14.4–97.4 kDa) (BioRad, Richmond, USA) or by the “Benchmark Prestained Protein Ladder” (10–170 kDa) (Gibco-BRL, Gaithersburg, USA). After gel electrophoresis, proteins were subsequently transferred to NC membranes (0.45 µm pore size, Protran BA 83, Schleicher & Schüll, Dassel, Germany) by semidry blotting at 2 mA/cm² for 30 min [18].

For protein staining, strips of the membrane were stained by AuroDye (Amersham Biosciences, Buckinghamshire, UK). For immunodetection, NC membranes were incubated in 0.1 M TBS (pH 7.4) containing 0.05% (v/v) Tween 20 (Sigma, St. Louis, USA) (TBST) for blocking free protein-binding sites. The membrane was then cut into strips, which were incubated with patients' sera or hybridoma cell culture supernatants (both diluted 1:20 in TBST) for detection of IgE-reactive components or specific antigens, respectively. The membrane strips were washed in TBST and incubated with the alkaline phosphatase-conjugated secondary antibody, monoclonal anti-human IgE (1:2000) (Allergopharma), or goat anti-mouse IgG/M (1:10 000) (Dianova, Hamburg, Germany). Binding patterns were visualized by a substrate solution containing 0.033% w/v nitroblue tetrazolium chloride (NBT) and 0.017% w/v 5-bromo-4-chloro-3-indolyl phosphate (BCIP), potassium salt (Sigma) in 0.1 M TBS, pH 9.5 [19].

2.4 2-DE and immunoblotting

2-DE was performed as previously described with slight modifications [20]. Briefly, IPG strips (Novex IPG Zoom Strips; Invitrogen, Groningen, Netherlands) with a pH range

of 3–10 were used for separation in the first dimension (IEF). Pollen extracts (100 µg) were loaded onto the gel strips for subsequent immunoblotting and 500 µg for protein sequencing. Separation was performed at 2 mA and 2 W with increasing voltage at 200 V for 20 min, 450 V for 15 min, 750 V for 15 min, and 2000 V for 1 h. SDS-PAGE was carried out at 200 V and 30 mA in the second dimension using gradient gels (Tris glycine Zoom gels 4–20%; Invitrogen). Molecular masses and pIs were determined by comparison with Multi Mark colored Standard (4–250.000 Da, Invitrogen) and IEF Marker 3–10, Liquid Mix (Serva, Heidelberg, Germany). Gels were silver stained for protein visualization [21].

For the identification of allergens, proteins were transferred by thermoblotting [22] from one gel onto several NC membranes to obtain replica and immunostained as stated above.

For the immunoblot inhibition assay, 1 mL of undiluted patients' pooled serum was preadsorbed by 10 mg maize or timothy grass pollen extract for 1 h at room temperature under agitation. After centrifugation, the preadsorbed serum was diluted 1:20 in TBST and used for immunostaining according to the standard procedure.

2.5 Protein sequencing

Proteins were transferred by semidry blotting onto PVDF membrane using 10 mM CAPS/10% methanol (pH 11.0) [23]. The membrane was then washed in double-distilled water, stained with 0.1% Coomassie (Serva) in 50% methanol, destained in 50% methanol and air-dried. Protein bands were excised and microsequencing was performed using a Procise protein sequencer connected to an online PTH amino acid analyzer (PE Biosystems, Weiterstadt, Germany).

2.6 Screening of a maize pollen cDNA library for allergens

A maize pollen cDNA library was constructed from maize inbred line A188 [24], according to the method described by Heuer *et al.* [25]. Briefly, a complementary DNA was cloned into λ-ZapXR Vector (Stratagene, La Jolla, CA) and then packaged into bacteriophages using Gigapack® III Gold Packaging Extract (Stratagene).

Library screening was performed with allergen-specific mAbs or by using of a DIG (digoxigenin)-labeled oligonucleotide to detect positive clones by plaque purification. The probe pPhlp1 (nucleotides 255–654) was constructed according to the manufacturer's description (PCR DIG Probe Synthesis Kit; Roche, Mannheim, Germany) by PCR with pPhlp1 (accession number Z27090) as a template.

pBluescript plasmid was excised *in vivo* from isolated phages by coinfection with helper phage R408 (Stratagene). Plasmids were amplified from single colonies, followed by plasmid preparation (Plasmid Purification Kit; Qiagen, Hilden, Germany). Insert analysis was carried out by *EcoRI/NotI* digestion and DNA sequencing.

3 Results

3.1 Comparison of maize and timothy grass pollen by Western blotting

We aimed at the determination of the maize pollen allergome and wished to compare it to that of native, wild-type grasses. Timothy grass was selected as a typical and well-characterized grass of temperate climates. We performed Western blotting followed by incubation with individual patient's sera and also classified the identified maize allergens by use of grass group-specific mAb.

As shown in Fig. 1A, Western blotting of maize pollen revealed a less complex allergen pattern compared to that of timothy grass pollen (Fig. 1B), showing just one intensive IgE-reactive band, possessing a molecular weight of 35 kDa, and an additional double band corresponding to a molecular weight of ~55 kDa. Protein bands of similar size were also detected in timothy pollen immunoblots. Using mAbs directed against known allergen classes, IgE-reactive maize pollen allergens were further characterized.

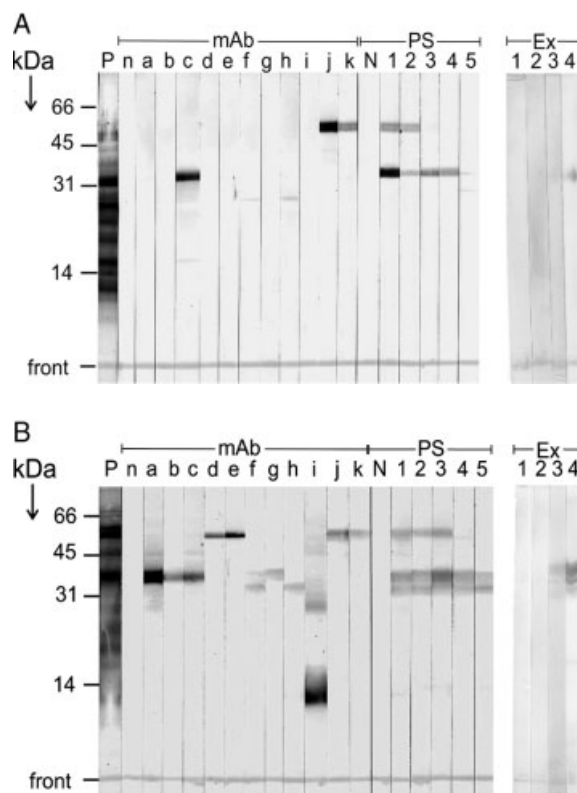


Figure 1. Comparison of maize (A) and timothy grass (B) pollen extracts by Western blotting using several mAbs (mAbs a–c, IG12, Bo14, and HB7 recognizing group 1 allergens; d–e, 3C4 and 5H1 → group 4; f–h, Bo1, BG6, and Bo9 → group 5; i, Bo12 → group 6; j–k, AF6 and EG9 → group 13) and some representative patient's sera (PS 1–5). P, protein staining by AuroDye; n and N, culture medium and serum of a healthy control; Ex 1–4, sera of maize pollen-exposed individuals.

One of the three mAbs directed against group 1 allergens in timothy grass pollen also bound to the previously detected 35 kDa maize pollen allergen, suggesting Zea m 1 identity. Both group 13 recognizing mAbs detected the 55 kDa component in maize pollen extract, suggesting protein Zea m 13 identity. In maize pollen extracts, none of the other highly immunogenic allergens of group 4, 5, and 6 present in timothy grass could be detected. The lack of group 4, 5, and 6 allergens is significant, because patients' sera 1–5 were determined as representative after a screening of 44 timothy grass pollen reactive sera. Only 65% of those sera bound to maize pollen extract. Interestingly, of all sera tested, 98% recognized Phl p 1 derived from timothy grass pollen, while only 64% recognized the corresponding Zea m 1, indicating a much lower IgE reactivity and/or crossreactivity of maize.

In a preliminary study, we diagnosed four individuals who were strongly exposed to maize pollen during their practical work in a botanical institute and displayed allergic reactions such as hayfever and itching after greenhouse work. As shown in Fig. 1, two of the sera showed neither IgE reactivity to maize nor to timothy pollen components (Ex 1–2), while the remaining Ex 3 and 4 bound to Zea m 1 and Phl p 1 proteins. Interestingly, both sera also recognized Phl p 5a and b, an allergen group that is known to be missing in maize pollen, suggesting that the sensitization was triggered by one of the common grass species, *e.g.*, timothy pollen.

3.2 Identification of maize pollen allergens by 2-DE and protein sequencing

To characterize the maize pollen allergens in more detail and to detect components possibly missed by mAb incubation of maize pollen extract, 2-DE blotting was performed and blots were subsequently incubated using IgE derived from pooled patients sera (a mixture of the five sera shown in Fig. 1).

As demonstrated in Fig. 2B, only some IgE-reactive proteins were detected, showing molecular weights of 55, 35, and 12 kDa. (The latter proteins correspond to multiple spots differing in their *pI*s.) In addition, a weak spot corresponding to a molecular weight of 20 kDa was seen. The proteins corresponding to a molecular weight of 35 kDa were distributed over a wide *pI* range and were identified as Zea m 1 by mAb HB7 (Fig. 2C). While the majority of the proteins focused at *pI* 8.0–9.0, two faint spots were detected at *pI* 4.2 and 4.9. The four protein spots at 55 kDa of *pI* 6.8–7.6 comprise Zea m 13 isoforms, as they were all recognized by the mAb AF6 (Fig. 2D).

Since none of the mAbs detected the marked IgE-reactive spots 3 and 4 (Fig. 2B), we performed protein sequencing of those spots using blotted allergens.

Protein microsequencing led to the unambiguous identification of all sampled allergens after an NCBI database search (Table 2). Zea m 1 identity of spots labeled 1 was

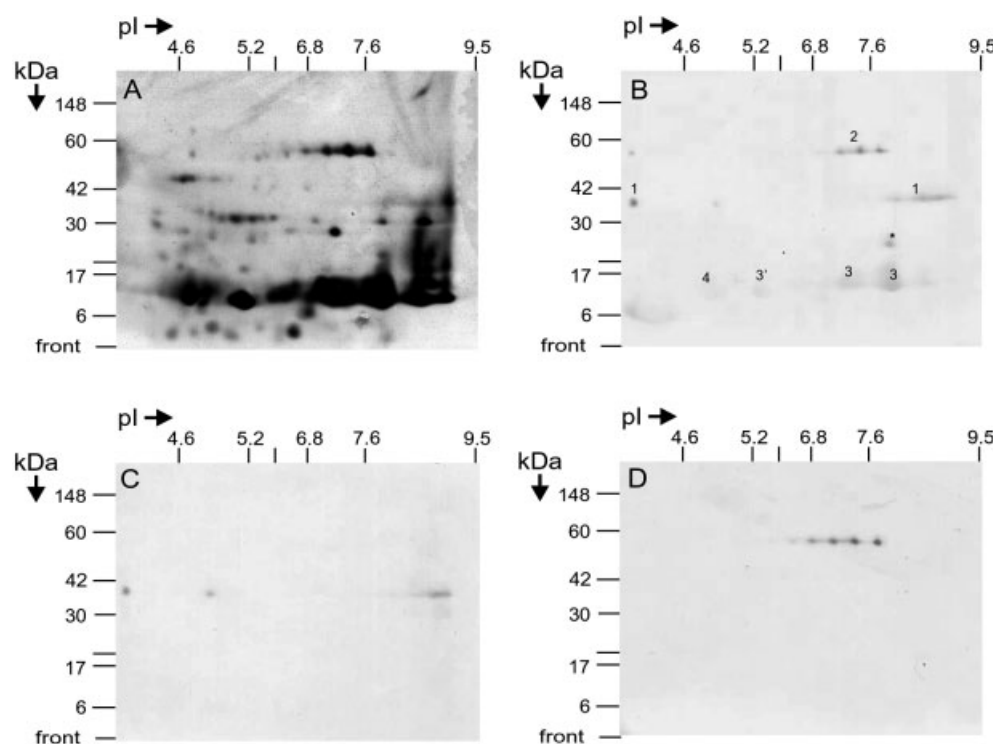


Figure 2. 2-DE and 2-DE immunoblotting of maize pollen extract. A, silver-stained gel; B, detection of IgE-reactive proteins by use of a patients' pooled serum; C, identification of grass group 1-specific allergens (mAb HB7); D, identification of grass group 13-specific allergens (mAb AF6). Numbered spots were determined by use of mAbs and by protein sequencing. 1, Zea m 1 isoforms; 2, Zea m 13 isoforms; 3, Zea m 3 isoforms; 3', acidic isoform of Zea m 3 or Zea m 2; 4, profilin; "*", not determinable IgE-reactive protein.

Table 2. N-terminal protein sequences obtained by analyzing the protein spots indicated in Fig. 2. A comparison of the protein sequences of both grass pollen allergen species are shown. Identical amino acid residues are highlighted. “*” Acidic isoform of Zea m 3 or Zea m 2

Spot	N-terminus	Allergen	Accession no.
1	GPPKVPPGP?IT	Zea m 1 Phl p 1	AY197353 Z27090
	GPPKVPPGPNIT		
	IPKVPPGPNIT		
2	EKEESKGID	Zea m 13 Phl p 13 *	X57627 CAB42886
	EKEESKGID		
	GKKEEKKEEKESG		
3'	GGALTFTVGKSGKPG		
3	TTPLTFTVGKSGKPG	Zea m 3 Phl p 3	AY331720 AY421969
	TTPLTFTVGKSGKPG		
	AVQVTFTVQKGSDDPK		
4	SWQTYVDE?LM?EI	Zea m 12 Phl p 12	X73279 X77583
	SWQTYVDEHLMCEI		
	SWQTYVDEHLMCEI		

confirmed and spots labeled 2 were identified as Zea m 13 isoforms. The fainter spots labeled 3 and 4 were identified as Zea m 3 and profilin (Zea m 12), respectively. While the spots labeled 3 completely matched the Zea m 3 protein sequence (NCBI accession no. AY331720), spot 3' revealed three amino acid exchanges at the N-terminus and may possibly be a Zea m 3 isoform. The protein amount of spot

“*” was too low for protein sequencing. As described in Table 2, the sequence identities of the N-termini vary considerably between the allergens of the two grass species. However, all maize allergens could be clearly identified after database searching.

After successful identification of crossreactive pollen allergens, we wished to compare the IgE binding/allergenicity of maize and timothy grass pollen allergens. This was accomplished by assessing residual IgE reactivity after preadsorption of patients' pooled sera with maize or timothy pollen extract on 2-DE blots. Figure 3 (upper row) shows binding to maize pollen after timothy grass pollen preadsorption. Figure 3C demonstrates that timothy grass pollen completely inhibits IgE binding to all 2-D-separated maize pollen components. This indicates the absence of maize-specific allergens and that timothy pollen covers all maize allergens. However, maize pollen extract only partly inhibits the IgE binding to timothy pollen components, as shown in Fig. 3E (lower row). Antibody binding to group 1 and 13 allergens of timothy pollen, comprising spots of about 35 and 55 kDa, was completely blocked, and IgE binding to group 3 (Phl p 3) and group 13 (Phl p 13) was considerably reduced. Reactivity to all other allergen spots, however, remained unchanged when compared to the ovalbumin-inhibited (negative) control. Figures 3B and F show that both pollen extracts completely inhibited the IgE antibodies from binding to the immobilized counterparts, serving as positive controls.

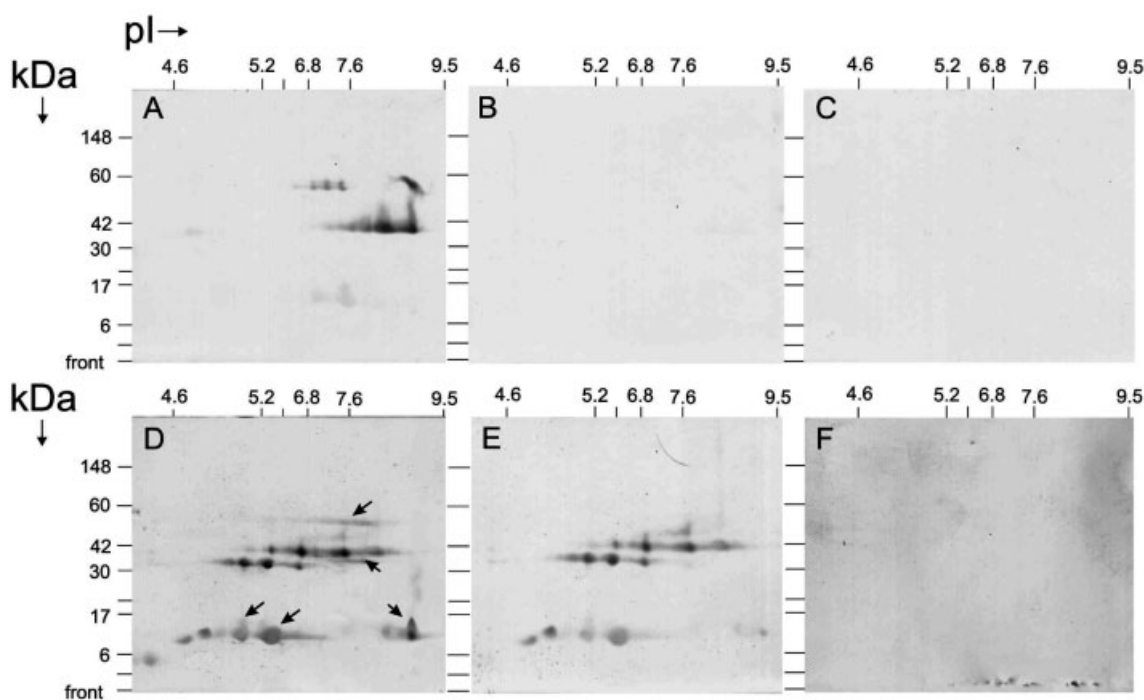


Figure 3. 2-DE immunoblot inhibition test for the identification of crossreactive allergens between maize and timothy grass pollen extract. A–C, 2-D-separated maize pollen extract; D–F, 2-D-separated timothy grass pollen extract. Patients' pooled serum without inhibitor (A, D), pretreated with maize pollen extract (B, E) and with timothy grass pollen extract (C, F).

3.3 Screening for Zea m 1 and Zea m 13 clones

Since Zea m 1 and Zea m 13 were identified as the most prominent allergens in maize, we next wished to study the structural variations among the isoforms and their probable influence on allergenicity. Sequences of three Zea m 1 proteins and one pZea m 1 clone (accession numbers #P58738, #AAO45607, #AAO45608, and #AY197353) and two pZea m 13 clones (accession numbers #X57627 and #X57628) were available from the NCBI database and were included in the protein sequence comparisons. In addition, a cDNA library of mature maize pollen was screened using different probes, and positive clones were isolated and sequenced to determine isoallergen variability. Furthermore, mAb AF6 was employed to screen for group 13 clones, while we screened for group 1 clones by hybridization employing a 435 kb DIG-labeled pPhlp1 oligonucleotide. Several attempts using mAb HB7 for screening had been unsuccessful before.

We obtained and analyzed two pZea m 1 clones and three pZea m 13 clones. Protein sequences of those showed a sequence identity of 72.1 and 69.9% if compared to timothy grass pollen allergens Phl p 1 and Phl p 13. Table 3 shows the allergen sequences of these novel clones, which have been submitted to the NCBI database (accessions #DQ421827; #DQ421829). The amino acid residues shown in bold type indicate variations among isoforms. Zea m 1 shows a higher sequence variability (39.7%, 95 amino acids/239 amino acids) than Zea m 13 (9.3%, 36/388 amino acids).

Computational analysis of the allergenic potential according to the Jameson and Wolf algorithm [26] was performed to assess allergenic potential of the maize allergens. Segments with an antigenic index >1.2 are underlined in Table 3. The antigenic regions were found to be distributed uniformly over the complete group 1 and 13 sequences of maize and timothy grass pollen. Some of those antigenic segments were found to be identical or partially overlapping between the two grasses, giving a possible explanation for the observed crossreactivity. However, unique antigenic regions were also predicted (as indicated in Table 3), suggesting differences in antigenicity between both grass species. In case of the group 12 and 3 allergens, we compared the protein sequences of the two grass species (Table 2). Group 12 allergens showed a highly conserved primary structure (80.6% amino acid identity), while group 3 allergens revealed only 34.5% amino acid identity.

4 Discussion

The aim of the present study was to examine the allergenic potency of maize pollen and to compare it to that of the native grass *P. pratense*. This was expected to possibly serve as a standard for assessing the influence of maize pollen on occupational asthma, e.g., for farmers or other strongly exposed persons, and also to assess the importance of altered

protein composition in genetically engineered maize varieties if compared to the cultivated wild-type maize.

Immunological and biochemical comparison showed that maize pollen contains only the four grass pollen allergen groups 1, 3, 12, and 13. Especially the high IgE prevalence of group 1 and 13 (Zea m 1 and Zea m 13) cause crossreactivity to grasses growing in temperate climate zones. Using sera of grass pollen allergic patients as well as highly maize pollen-exposed individuals, we did not identify any maize-specific IgE-reactive component. Analyzing four sera of maize pollen-exposed individuals by Western blotting, we determined IgE reactivity to the group 1 allergens and a positive skin test to maize pollen extract in two of them. Since IgE binding to the group 5 allergens, which is lacking in maize pollen, was detected in both individuals, the sensitizing pollen was probably derived from a different grass species.

The structural differences found in maize and other grass species reflect the fact that maize belongs to the Panicoideae, while European native grasses belong to the same grass subfamily Pooideae [5]. Although cultivated for a long time, exclusively maize allergic patients were very rarely reported [27, 28] even though the crossreactive proteins are homologous to allergenic grass pollen proteins. We identified IgE crossreactivity of the group 12 allergens (profilins) in maize, which showed a high sequence identity between both plant species of 80.6%. Because of their fundamental role in the formation of the cytoskeleton, profilins are highly conserved proteins; however, profilin IgE reactivity is weak (IgE prevalence for timothy pollen amounts to just about 10% [29]). Zea m 1 represents an expansin, involved in the stretching of cell walls and in the growth of pollen tubes for fertilization [30]. In spite of the important function, the structural variability of Zea m 1 between timothy and maize is high [31, 11], which is also the case among Zea m 1 isoforms. In grasses, the IgE prevalence of 95% as determined in the case of Phl p 1 indicates that this protein represents the single most important grass pollen allergen [32]. The group 13 allergens were identified to function as polygalacturonases [33, 34]. These allergens are highly conserved, showing only few amino acid exchanges. Especially, the catalytic site consisting of amino acid residues 288–300 (in maize) is highly conserved. The group 3 allergens differ considerably in their protein sequences and reveal only 34.5% sequence identity (accession number #AY331720) [35]. N-terminal sequencing of the Zea m 3 isoform at pI 4.5 (3') shows additional amino acid differences. The acidic pI and the partial sequence similarity to group 3 allergens might also indicate that the identified protein actually belongs to grass group 2, which has not been identified in maize so far. However, both allergen groups have been detected and characterized in ryegrass (*Lolium perenne*), orchard grass (*Dactylis glomerata*), and timothy grass and reveal sequence identities of about 50% and an acidic or alkaline pI [35]. Both allergens were also reported to belong to the expansin group [36] and both show sequence similarity

Table 3. Comparison of the protein sequences between maize and timothy grass pollen. For grass group 1, we compared Zea m 1-clone (NCBI accession #P58738) and Phl p 1 (#Z27090); for grass group 13, Zea m 13-clone (#DQ421829) and Phl p 13 (#AJ238848). Regions of high antigenicity (antigenic index >1.2) are underlined. Differences in the protein sequences of the maize allergen isoforms are indicated in bold (details are given in the text)

Zea m 1	GPPKVPPGKNITATY <u>GKDWLDAKATWY</u> GKPTGAGPDDNGGGCGYKDVNKP <u>PFNSMGACGNI</u>
Phl p 1	IPKVPPGPNITATY <u>GDKWLDKSTWY</u> GKPTAAGPKDNGGACGYKDVDKPP <u>ESGMTGCGNT</u> ***** ***** . : ***** : ***** . *** . **** . ***** : ***** . * . ***
Zea m 1	PIFKDGLGCGSCFE <u>IKCDKPVECSGK</u> FVVVHITDMNYEPIAAYHFDLAGTAF <u>GAMAKKGE</u>
Phl p 1	PIFKSGRGCGSCFE <u>IKCTKPEACSGE</u> FVVVHITDDNEEPIAAYHFDLSGIAFG <u>SMAKKGD</u> **** . * ***** ***** ** *** : ***** * ***** : * *** : ***** :
Zea m 1	EEKLRKAGI <u>IDMQFRRVKCKFGS</u> --KVSFHLEKGG <u>ENYLALLVKYVDGDGDIVPVD</u> <u>IKE</u>
Phl p 1	EQKLRSAGEVEIQFRRVKCKY <u>PEGTKVTFHVEKGSN</u> <u>ENYLALLVKFVAGDGDVVAVDIKE</u> * : *** . ** : : ***** : . ** : ** : *** . ***** : * ***** : . *****
Zea m 1	<u>KGSDTYEPLKHSWGAIWRKDSDKP</u> <u>LKGPLTVRLTTEGGT</u> <u>KTIVYDDVI</u> <u>PANWKANTAYTAK</u>
Phl p 1	<u>KGKDKWIALKE</u> <u>SWGAIWRIDTPEVLKGPFTVRYTTEGGT</u> <u>KGEAKDVI</u> <u>PEGWKADTAYESK</u> ** . * . : . ** . ***** * : : ***** : *** ***** . ***** . *** : *** : *
Zea m 13	-----EKEES <u>KGIDAKASGPGGFFDITKL</u> <u>GASNGKTDSTKAVQEA</u> <u>WASACGGTGKQITIL</u>
Phl p 13	GKKEEKKEEKES <u>SGDAASGADGYDITKL</u> <u>GAKPDPGKT</u> <u>DCTKEVEEAWASACGGTGKNTIV</u> : *** . * . *** . * : ***** . : ***** . * * : ***** : *** :
Zea m 13	IPKGDFLVGQLNFTGPCKGD <u>-VTIQVDGNLLATTDLSQYKEHGNWIEILRVDNLVITGKG</u>
Phl p 13	IPKGDFLTGPLNFTGPCKGDSVTIKLDGNLLSSNDLAKYKAN <u>--WIEIMRIKKLTITGKG</u> ***** . * ***** ***** *** : ***** : . ** : ** : ***** : * : . *****
Zea m 13	<u>NLDGQGP</u> <u>AVWSKNFCTKKYDCKIL</u> <u>ENSLVMDFVNNGEVSGITL</u> <u>NSKFFHMNMYQCKNMI</u>
Phl p 13	<u>TLDGQGK</u> <u>AVWGKN</u> <u>SCAKNYNCKIL</u> <u>PNTLVLD</u> <u>FCDDALIEGITL</u> <u>NAKFFHMNIYECKGVT</u> . ***** *** . ** * : * : ***** : ** : ** : . . : ***** : ***** : * : * . :
Zea m 13	IKDVTVTAPGDS <u>PNTDGIHMGDS</u> <u>SGITITNTVIGVGD</u> <u>CISIGPGTSKVNI</u> <u>TGVT</u> <u>CGPGH</u>
Phl p 13	VKDVTITAPGDS <u>PNTDGIHIGDS</u> <u>SKVTITDTTIGTGDDCISIGPGSTGLNI</u> <u>TGVT</u> <u>CGPGH</u> : ***** : ***** : ***** : *** : *** : * . ** . ***** : : : *****
Zea m 13	<u>GISIGSLGRYKDEKDVRDINVKDCT</u> <u>LKKT</u> <u>MFVRIKAYEDAASVLT</u> <u>TVSKIHYENIKMEDS</u>
Phl p 13	<u>GISVGS</u> <u>SLGRYKDEKDVT</u> <u>DITVKN</u> <u>CVLKKSTNGLRIKSY</u> <u>EDAKSPLT</u> <u>ASKLTYENVK</u> <u>MEDV</u> *** : ***** ***** ** . ** : * . *** : * : *** : ***** * ** . ** : *** : *****
Zea m 13	ANPIFIDMKYCPNKLCTANG <u>-ASKVTVKDITFKNITGT</u> <u>SSTPEAIS</u> <u>LLCTAKVPCT</u> <u>GVTM</u>
Phl p 13	GYPIIIDQKYCPNKICT <u>SKGDSARVTVKDVTFRNITGT</u> <u>SSTPEAVS</u> <u>LLCSDKQPCNGVTM</u> . ** : ** ***** : *** : * : : ***** : ** : ***** : ***** : * ** . *****
Zea m 13	DDVNVEY <u>SGTNNKTMAICTNAKGST</u> <u>KGCLKELACF</u> -
Phl p 13	NDVKIEY <u>SGTNNKTMAVCTNAKV</u> <u>TAKGVSEANTCAA</u> : * : : ***** : ***** : : ** : : *

to the C-terminal part of group 1 allergens. Since no IgE-reactive epitope could be identified for Phl p 3 and Phl p 2 by peptide mapping, we assume the presence of conformational epitopes in those allergens [35].

Interestingly, we did not identify allergens belonging to the lipid transfer proteins and albumins/globulins, which were determined as major allergens in maize flour [37, 38]. Probably, these allergens are not expressed in pollen or they might not have been detected by patients' sera from the northern part of Germany. Our results correspond to the data of Sander *et al.* [39], who also determined different allergens as elicitors of grass pollen allergies and occupational baker's asthma. Thus, according to our data, only two prominent and two less important crossreactive allergens may be assumed to mainly cause the allergenicity of maize. Two structural characteristics may explain the low incidence of maize allergenicity if compared to pollen of other grasses. First, increased pollen size of the grains (Fig 4) causes a low aerial spread of maize pollen. In comparison, maize pollen, although having a shape similar to European native grass pollen, is about three times larger in diameter and thus possesses some 27-fold higher volume and probably weight than grass pollen. Second, pollen grains of maize are more sticky due to pollen kitt present on the exine surface, which causes agglomeration of the pollen and may prevent aerial spread even further [40]. However, pollen fragments, such as starch granules, may be released and distributed as published by Suphioglu *et al.* [41], which may be of importance considering the tremendous amounts of maize pollen produced in agriculture. This may slightly increase pre-existing grass pollen allergies in susceptible patients.

From these results, we conclude that maize pollen plays a less important role as a sensitizer when compared to pollen of our common grass species. This may be explained by morphological differences, as well as the apparently lower allergen content and the lower number of allergen groups found in maize pollen.

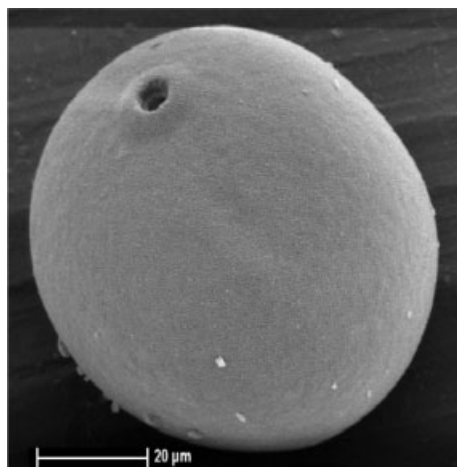


Figure 4. Maize pollen shown by raster electron microscopy. The size of the bar is indicated.

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5 References

- [1] Schubert, H. J., Jäger, L., Rudeschko, O., *Allergojournal* 2005, 14, 209–213.
- [2] Lehrer, S. B., Reese, G., Malo, J. L., Lahoud, C. *et al.*, *Int. Arch. Allergy Immunol.* 1999, 118, 298–299.
- [3] Stewart, G. A., Thompson, P. J., *Clin. Exp. Allergy* 1996, 26, 1020–1044.
- [4] Andersson, K., Lidholm, J., *Int. Arch. Allergy Immunol.* 2003, 130, 87–107.
- [5] Weber, R. W., *J. Allergy Clin. Immunol.* 2003, 112, 229–239.
- [6] Petersen, A., Schramm, G., Becker, W. M., Schlaak, M., *Biol. Chem. Hoppe Seyler* 1993, 374, 855–861.
- [7] Behrendt, H., Becker, W. M., *Curr. Opin. Immunol.* 2001, 13, 709–715.
- [8] Valenta, R., Lidholm, J., Niederberger, V., Hayek, B. *et al.*, *Clin. Exp. Allergy* 1999, 29, 896–904.
- [9] Jutel, M., Jäger, L., Suck, R., Meyer, H. *et al.*, *J. Allergy Clin. Immunol.* 2005, 116, 608–613.
- [10] Becker, W. M., Schaubschläger, W., Westphal, W., Schlaak, M., in: Chmiel, H., Hammes, W. P., Baily, J. E. (Eds.), *Biochemical Engineering*, Stuttgart, Fischer 1988, pp. 440–442.
- [11] Petersen, A., Schramm, G., Bufer, A., Schlaak, M., Becker, W. M., *J. Allergy Clin. Immunol.* 1995, 95, 987–994.
- [12] Petersen, A., Schramm, G., Kramer, A., Grobe, K., Becker, W. M., *Allergy Clin. Immunol. Int.*, in press.
- [13] Blume, C., Lindner, B., Becker, W. M., Petersen, A., *Proteomics* 2004, 4, 1366–1371.
- [14] Petersen, A., Suck, R., Hagen, S., Cromwell, O. *et al.*, *J. Allergy Clin. Immunol.* 2001, 107, 856–862.
- [15] Fahlbusch, B., Müller, W. D., Diener, C. H., Jäger, L., *Clin. Exp. Allergy* 1993, 23, 51–60.
- [16] Fahlbusch, B., Müller, W. D., Rudeschko, O., Jäger, L. *et al.*, *Clin. Exp. Allergy* 1998, 28, 799–807.
- [17] Laemmli, U. K., *Nature* 1970, 22, 680–685.
- [18] Kyhse-Andersen, J., *J. Biochem. Biophys. Methods* 1984, 10, 285–289.
- [19] Leary, J. J., Brigati, D. J., Ward, D. C., *Proc. Natl. Acad. Sci. USA* 1983, 80, 4045–4049.
- [20] Görg, A., Postel, W., Günther, S., *Electrophoresis* 1988, 9, 513–546.
- [21] Heukeshoven, J., Dernick, R., *Electrophoresis* 1988, 9, 28–32.
- [22] Jägersten, C., Edstrom, A., Olsson, B., Jacobson, G., *Electrophoresis* 1988, 9, 662–665.
- [23] Matsudaira, P., *J. Biol. Chem.* 1987, 262, 10035–10038.
- [24] Green, C. E., Phillips, R. L., *Crop. Sci.* 1975, 15, 417–421.

- [25] Heuer, S., Lörz, H., Dresselhaus, T., *Sex. Plant Reprod.* 2000, **13**, 21–27.
- [26] Jameson, B. A., Wolf, H., *CABIOS* 1988, **4**, 181–186.
- [27] Gonzalo-Garijo, M. A., Perez-Calderon, R., Munoz-Rodriguez, A., Tormo-Molina, R., Silva-Palacios, I., *Allergy* 2004, **59**, 365.
- [28] Freeman, G. L., *Ann. Allergy* 1994, **72**, 415–417.
- [29] Valenta, R., Duchene, M., Ebner, C., Valent, P. *et al.*, *J. Exp. Med.* 1992, **175**, 377–385.
- [30] Cosgrove, D. J., Bedinger, P., Durachko, D. M., *Proc. Natl. Acad. Sci. USA* 1997, **94**, 6559–6564.
- [31] Li, L. C., Bedinger, P. A., Volk, C., Jones, A. D., Cosgrove, D., *J. Plant Physiol.* 2003, **132**, 2073–2085.
- [32] Laffer, S., Spitzauer, S., Susani, M., Pairleitner, H. *et al.*, *Allergy Clin. Immunol.* 1996, **98**, 652–658.
- [33] Niogret, M. F., Dubald, M., Mandaron, P., Mache, R., *Plant Mol. Biol.* 1991, **17**, 1155–1164.
- [34] Suck, R., Petersen, A., Hagen, S., Cromwell, O. *et al.*, *Clin. Exp. Allergy* 2000, **30**, 324–332.
- [35] Petersen, A., Suck, R., Lindner, B., Georgieva, D. *et al.*, *Clin. Exp. Allergy* 2006, **36**, 840–849.
- [36] Cosgrove, D. J., *Gravit. Space Biol. Bull.* 1998, **11**, 61–70.
- [37] Pastorello, E. A., Farioli, L., Pravettoni, V., Ispano, M. *et al.*, *Allergy Clin. Immunol.* 2000, **106**, 744–751.
- [38] Baldo, B. A., Wrigley, C. W., *Clin. Allergy* 1978, **8**, 109–124.
- [39] Sander, I., Raulf-Heimsoth, M., Duser, M., Flagge, A. *et al.*, *Int. Arch. Allergy Immunol.* 1997, **112**, 378–385.
- [40] Suen, D. F., Wu, S. S., Chang, H. C., Dhugga, K. S., Huang, A. H., *J. Biol. Chem.* 2003, **278**, 43672–43681.
- [41] Suphioglu, C., Singh, M. B., Taylor, P., Bellomo, R. *et al.*, *Lancet* 1992, **339**, 569–572.