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Cereal pollen sensitisation in pollen allergic patients: to treat or not to treat?

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Summary

Pollen allergens of the Poaceae family comprise one of the main causes of pollinosis worldwide. Although most of cereals are included in this family, certain pollination characteristics and aerobiological features differentiate them from common wild grass pollen. Cereal pollen grains cannot be easily characterised as potential sources of aeroallergens, because of their pollination mode (most of them are autogamous plants), the consecutive low numbers of pollen they produce and their pollen's large volume and consequent high weight which further prevent dispersion and pollen transport. However, various epidemiological studies concluded in comparable sensitisation patterns between common grass and cereal pollen. This fact can be attributed to the common epitopes shared between grass and cereal pollen allergens, which are responsible for the high cross reactivity observed not only in vitro but also in vivo. Therefore, the sensitisation patterns do not usually reflect a genuine, cereal-specific IgE recognition. On the contrary, genuine cereal sensitisation and allergy are referred to only in rare cases of occupational exposure to high amounts of these pollen grains (farmers and field workers) or in patients staying in the vicinity of cereal fields. Thus, cautious considerations should be taken into account when diagnostically approaching patients in which cereal pollen allergy could be considered in the differential diagnosis.

Introduction

The family of Poaceae (grasses) comprises one of the largest plant families regarding the number of representative taxa it includes (1, 2). It consists of both annual and perennial herbs, with many species being cosmopolitan and therefore being frequently found in many different locations, at varying latitudes and geoclimatic zones, in and around urban areas (3). The Poaceae family also includes many of the cereal species being cultivated worldwide. Cereal crops are economically the most important in the world, as they comprise a principal food source in many societies (4). In this paper, we review the majority of cereal taxa belonging to the Poaceae family; those few which do not belong to this family, like buckwheat (*Fagopyrum esculentum*, family Polygonaceae), grain amaranth (*Amaranthus cruentus*, family Amaranthaceae) and quinoa (*Chenopodium quinoa*, family Chenopodiaceae) (5), are not currently reviewed.

The main cereal taxa are analysed from an ecological, agricultural and aerobiological perspective. Epidemiological, clinical and allergic features are also discussed in order to reveal cereal pollen impact on grass-pollen sensitisation patterns and conclude in a state-of-the-art diagnostic approach and treatment.

Ecological and agricultural characteristics of cereals

Certain cereals were domesticated and brought into cultivation by the Neolithic people, primarily in Asia (5). Since then, they have been spread widely around the world and can be found in all continents, regardless of climate, atmospheric and soil properties and latitude.
Nowadays, they can be grown almost everywhere, especially after the creation and production of genetically modified and enhanced varieties of specific species and better-adapted hybrids (i.e. triticale) or after the application of intensive and human-controlled agricultural techniques. Thus, over the first decade of the 21st century, maize, wheat and rice together accounted for 80-90% of all grain production worldwide, and almost 40% of calorie intake as a whole (5).
As a rule, cereals are significantly influenced by environmental factors, like water availability, nutrient levels and air temperature. The warm-season cereals (rice, sorghum) prefer hot weather, thus frequently grown in tropical lowlands year-round and in temperate climates during the frost-free season. Cool-season cereals (wheat, rye, triticale, oats and barley) are well-adapted to temperate climates, although different varieties of particular species could be either winter or spring types. Winter varieties are sown in the autumn, germinate and grow vegetatively, then become dormant during winter and do not flower until spring. Spring cereals are planted in early springtime, flower and mature later the same summer.

Flowering and aerobiology of cereals

Flowering

Flowering of cereal taxa, and concomitantly pollen production, dispersion and atmospheric circulation, depends on various environmental factors, the most important of which are air temperature, rainfall and relative humidity (6-9). In general, representatives of the Poaceae family flower from early spring (March-April) to end of summer (July-August). Cereal taxa mainly flower during spring (2, 3, 6) and usually earlier than the wild, non-cultivated species of Poaceae.

Pollination

Anemophilous plant taxa produce large quantities of pollen grains (to compensate for their low pollination efficiency) which are dispersed and transported with the wind (6, 10)[1]. Cereal taxa sometimes consist of plants that are not strictly anemophilous, unlike the rest representatives of the Poaceae family.
Some cereals, among other plant taxa, are often self-pollinated or autogamous, that is no pollen is exposed (the male reproductive organ of a plant releases its pollen grains into the female organ of the same plant). In this case, very little pollen is produced and only accidentally this becomes airborne (10). This contradicts the pollination of wild (not cultivated) grasses, which, in most cases, present a high number of spikelets (analogous to flowers), which usually produce high quantities of pollen grains (2). For pollen dispersion, weather variables and particularly wind components (direction, speed and persistence) are also very important (8).
In Table 1, a list of the main cereal taxa is shown, with the main representative species and their common names, along with their – primary – pollination mode. It is clear that out of the 14 cereal taxa reviewed in this paper, seven are self-pollinated and only four are considered as strictly anemophilous.

Aerobiology

Aerobiology of allergenic pollen is usually studied by use of volumetric (Hirst-type) devices, which can trap airborne particles that thereafter are microscopically identified, counted, and finally expressed as atmospheric pollen concentrations (12). Grass pollen grains can be technically identified under a light microscope only at the level of family (10, 13). Grasses, as a group, produce spheroidal to ovoidal, monoporate pollen grains, with a granular surface (Figure 1). It is, therefore, not possible to differentiate among species or even genera. Attempts have been made to distinguish between specific taxa, particularly cereals (i.e. *Zea mays*, *Oryza sativa*) based on their larger pollen grains (the sizes of the main cereal taxa are shown in Table 1). However, it does not seem feasible to recognise even the members with the largest pollen, like corn and barley.
Even when using the criterion of pollen size or taken into account phytosociological and population ecology

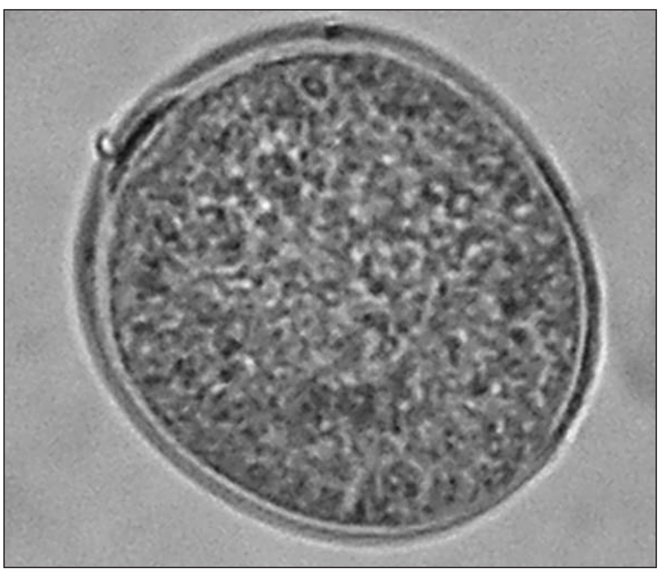
[1] In contrast, zoogamous (animal-pollinated) i.e. *entomophilous* taxa, base their reproductive success on animal vectors i.e. insects: pollen grains are carried from the anther of one flower (male reproductive organ) to the stigma of another by insects, birds, bats etc. Zoogamous taxa shed less pollen, with the latter becoming airborne only occasionally. Intermediate pollination modes also exist, where pollination is achieved both by wind and animal vectors, and then plant taxa are characterised as *ambophilous* (11).

Table 1 - Taxa (genera, most representative species and common names), pollination mode and pollen size of the main cereals.

Taxon*,+				
Genus	Species	Common name	Pollination mode+	Size of pollen grains (in μm)+
Avena	*A. sativa*	Oats	SP	48-66
Digitaria	*D. exilis*	Fonio	SP	32-45
Eragrostis	*E. tef*	Teff	SP	n/a
Hordeum	*H. vulgare*	Barley	SP	86-122
Oryza	*O. sativa*	Rice	SP	n/a
Panicum	*P. maximum* *P. miliaceum* *P. mole*	Millet	n/a	32-45
Pennisetum	*P. glaucum*	Pearl millet	n/a	32-45
Secale	*S. cereale*	Rye	SA	52-65
Setaria	*S. italica*	Foxtail millet	SA	32-45
Sorghum	*S. bicolor*	Sorghum	SP	32-45
Triticum	*T. aestivum* *T. durum* *T. sativum*	Bread wheat Durum wheat	SP	40-122
Triticum x Secale	*hybrid*	Triticale	PA	40-122
Zea	*Z. mays*	Corn	SA	86-122
Zizania	*Z. aquatica*	Wild rice	SA	32-45

SP: self-pollinated, PA: partly anemophilous, SA: strictly anemophilous, n/a: not acknowledged
**: After van Wyk (5), +: After Lewis et al. (2)*

Figure 1 - Poaceae pollen grain, as observed under light microscope



studies, discrimination among different pollen types is actually unlikely. In addition to that, pollen size is highly variant depending on the chemical substances (i.e. glycerol) used for the preparation of slides, and also related to the absorption efficiency of pollen per taxon. Moreover, the Poaceae family is a diverse family, with a high number of species and with plant communities whose ecology allows them to be found at many different habitats all over the world. Likewise, the criterion of flowering phenology of different grass species and its comparison with airborne pollen levels of the Poaceae family is also not reliable, as there is usually overlapping of many species, virtually being highly unlikely to discriminate among them (13).

Very few studies have tried to identify the species, among the numerous included in the Poaceae family, which actually contribute to the total airborne pollen concentration of grasses. In a study conducted in Spain, León-Ruiz et al. (4) suggested that only four (*Dactylis glomerata*, *Lolium rigidum*, *Trisetaria panicea* and *Vulpia geniculata*) were the main pollen-producing Poaceae species among 33 different Poaceae species considered to be the most highly represented in the regional vegetation in Spain (14). Similarly, Fotiou et al. (15) found that pollen production rates of the self-pollinated Hordeum murinum (commonly known as wall barley or false barley) are rather low and is a very low contributor to the total Poaceae pollen count of the study area (Thessaloniki, Greece); therefore, it is not considered as a potential cause of pollination, even though this particular species is the most abundant representative of all grasses in the study area.

Sensitisation and allergic diseases to cereal pollen

Factors influencing potential sensitisation and sources of cereal pollen

The wide distribution of grass species in general, together with the high allergenicity, explains why these species comprise the main cause of pollinosis not only in Europe (7, 9, 16, 17), but also worldwide (2, 18, 19). Epidemiological studies have revealed that, in many circumstances, more than 50% of the atopic individuals are sensitised to non-cultivated grass and/or to cereal pollen (18-28), however after utilising heterogeneous samples of asymptomatic or allergic individuals. Nevertheless, cereal pollen grains cannot be easily characterised as potential sources of aeroallergens, because of their pollination mode (most of them are autogamous plants), the consequent low numbers of produced pollen, and their pollen's large size. Therefore, exposure to pollen allergens is considered negligible for self-pollinated cereal taxa in general, while cases with documented respiratory allergies to cereal pollen are in the form of occupational allergy, observed mainly in people working in areas where exposure is inevitably high. This is also the case with strictly anemophilous cereal taxa, like maize, rye and foxtail millet (Table 1); their pollen, although they are produced in copious amounts, are of large size (and concomitantly of high weight), therefore making their dispersion and transport possible for a distance of no more than 0.5 km (2). On these grounds, allergenic importance of such cultivated grasses (like *Triticum, Secale, Hordeum*) is also diminished, as they may sensitise and cause allergic symptoms only to people directly exposed to these fields (i.e. farmers), and thus are considered as occupational allergens (6). This could be also the case for individuals living in the vicinity. Moreover, they may also trigger symptoms to individuals already sensitised to non-cultivated grass-pollen due to their significant cross-reactivity.

Maize pollen comprises a very well studied example. Its pollen grain has a size of 86-122 μm and has about three times larger diameter comparing with the common grass pollen grains; thus, it has an estimated 27-fold higher volume and consequently higher weight. In addition to that, the sticky pollen coat material on their exine surface due to pollenkitt (adhesive material present around pollen grains of almost all angiosperms), may cause agglomeration of multiple grains, in that way increase volume and weight and further prevent dispersion and pollen transportation (29). These facts explain why only a very small percentage of maize pollen actually becomes airborne and only 5% could be dispersed more than 60 meters by wind (30), even though maize pollen production is prolific with an average-sized tassel (the male inflorescence) producing approximately 25 million pollen grains (31). As hybrids have been bred for closer spacing, tassel size and pollen production have been found to decrease (32). Therefore, corn pollen could only be considered as an aeroallergen provoking occupational allergy and should be treated as such, since neither does it shed pollen into greater distances nor its pollen grains remain viable for long time (2).

Unfortunately there are only few studies in which this self-explanatory conclusion has been shown. Valencia Zavala et al. (33) examined the role maize pollen has in patients with respiratory allergies, and found it to be highly relevant in workers of maize, although as a nonspecific sensitisation may occur in other patients as well. Similarly, Sen et al. (18) documented a high concentration of airborne rice pollen in rice crops during rice flowering, a bimodal diurnal patter of pollen release and positive skin-prick tests with the antigenic extract of the rice pollen in patients with respiratory symptoms during rice flowering period. All the above indicate the risk of rice pollen allergy among agricultural field workers as well as the people staying in the vicinity of rice fields.

Cereal pollen can be a source of allergens and a cause of allergies not only in agricultural field workers but also in other occupations. Iversen and Pedersen (34) have observed sensitisation to cereals in Danish farmers. The study demonstrated that exposure to these pollen grains in cowsheds is massive, is recorded all year round and that by far exceeds outdoor concentrations. A similar rise in pollen concentrations associated with cattle feeding has also been observed inside dairy farms, in France, Germany and Switzerland (35).

Grass and cereal pollen allergens' cross reactivity

Kalveram and Forck (36) tried to assess the analogous skin prick test reactions of grass and corn pollen extracts in allergic patients. In this study, grass pollen showed weak inhibition with rye, wheat and barley. This was also the case with corn pollen which showed little cross-reactivity. On these grounds, immunobiological studies have shown that members of the sub-family Panicoideae, in which corn belongs, lack allergens of the groups 2 and 5. This explains the little cross-allergenicity with members of Pooideae, like timothy grass, sweet vernal grass, ryegrass and orchard grass, which have group 1, 2/3 and 5 major allergens (37).

According to these observations, Kalveram and Forck (36) suggested that members of this taxon must be tested and treated separately. However, in the same study (36), grass pollen antigens appeared to strongly inhibit all cereal antigen-antibody reactions suggesting that the exclusive use of the studied mix-grass pollen extract could safely replace individual cereal extracts in diagnosis and perhaps in therapy of combined grass and corn pollen allergy.

In another study for maize pollen, proteome analysis for allergy relevant components has revealed that timothy pollen extract completely blocks IgE binding to maize, while maize pollen extract blocks IgE reactivity to only some timothy pollen allergens, reinforcing the observation of the previous study. The two prominent allergens from maize were found to be Zea m 1 and Zea m 13 which cross-react with Phl p 1 and Phl p 13 from the timothy grass pollen allergen groups (38). Thus, cereal pollen sensitisation cannot be reliably proven by using cereal pollen extracts, since they contain epitopes that are similar to grass pollen allergens. Genuine cereal pollen sensitisation can be ensured only at a molecular level. Again the similarities between corn and timothy major allergens can justify the use of timothy as the main diagnostic extract for skin prick testing.

Weeke and Spieksma (9) have suggested that as both flowering and pollen seasons of wild grasses and cultivated cereals are so closely related, regardless of the species examined, it is not possible to select only one species for the allergy testing procedure. However, in a Danish multicentre study of 5,000 patients (12), it was observed that because of cross-reactivity between the different grass pollen sub-families and genera, 75% of all grass-allergic individuals could be identified by using timothy grass skin prick test extract alone, which, however, is considered the major representative of all grasses. Thus, although timothy can probably replace cereal extracts in diagnostic procedures, it cannot diagnostically substitute grass pollen allergen extracts of families with little cross reactivity and cross allerginicity (e.g. bermuda grass belonging to Chloridoideae sub-family) (39).

Among cereal pollen allergens, rice pollen showed no cross-reactivity with corn pollen and weak cross-allergenicity with wheat, orchard, and timothy pollen (40).

From sensitisation to clinical syndromes and treatment

According to several studies, cereal pollen sensitisation is ranked among the most prevalent (41-43). In the majority of these studies, sensitisation has been usually approached by skin prick testing patients with respiratory symptoms using extracts from mixtures of cereal pollen grains, or even mixtures of cereals and Poaceae pollen in general (44-46). This high frequency can be ascribed to cross-sensitivity between wild grass and cereal pollen which can also explain why cultivated maize, rice, wheat, rye, barley and oats, can also trigger allergic symptoms in individuals sensitised to grass pollen.

Nevertheless, cereal crop pollen sensitisation plays a minor role in patients sensitised to aeroallergens, as a whole. In a recent survey of almost 20,000 patients, only 0.2% appeared to have a clinically related sensitisation to cereal pollen (47). But even in cases where sensitisation rates to cereal pollen are high (48), it is not actually proven or even hinted that clinically relevant allergy to cereal pollen can be of high frequency and thus comprise a health risk.

This is not the case in occupationally exposed individuals who can be directly susceptible to cereal pollen allergens or may indirectly have a significant occupational hazard if sensitised to common cross-reactive grass pollen, such as *Poa, Phleum pratensis* and *Lolium* (49). Below, the clinical aspects of some of the most widely cultivated cereal species are quoted.

Oat (Avena sativa)

Sensitisation to oat pollen ranges from 11.3% to 46.9% (16, 48, 50). Cultivated oat pollens do not have distinctive allergens, however, they share common allergens with grass groups 1, 2 and 5 (51, 54).

Interestingly, oat pollen has been characterised as an important occupational source of allergens among dairy farmers in Finland (52).

Barley (Hordeum vulgare)

Barley pollen expresses multiple isoforms of specific allergens, designated as Hor v 9, taking also into account that it has the same group of *Poa pratensis* pollen allergen Poa p 9 (49, 53).
Sensitisation to barley pollen can reach up more than 50% (48). Positive skin prick tests to barley and rye were found to coexist in up to 80% in French children, who were sensitised also to wild grass pollen with respiratory symptoms during summer months. Although potential cross-reactivity was mentioned, clinical-cross reactivity was not tested (54).

Rice (Oryza sativa)

Sensitisation to rice pollen ranges from 9.3% to 67.1% (20). A study conducted in India (18) provided clear aerobiological and clinical evidence, suggesting that rice pollen allergens could be the offending allergens for the agricultural field workers as well as the people staying in the vicinity of the rice fields. Skin prick tests were performed in 240 patients who had clinical history to pollen allergy and who reported higher symptoms of respiratory allergy during the rice flowering periods. 66% of them were found to be positive to pollen allergen extract of *Oryza sativa* (var. Ratna), whereas cross-reactivity was found with the pollen allergens of other rice varieties and with the wild grass *Phragmitis karka*.

Rye (Secale cereale)

Cultivated rye grass is different from ryegrass (*Lolium perenne*). At least nine major allergens belonging to grass groups 1, 2 and 5 have been determined (37, 55) and two [Sec c 5 (56) and Sec c 4 (57)] have been well characterised.
Different studies have shown sensitisation levels to rye pollen ranging from 27.7% to 90.0% (22-24, 48). In a paediatric cohort in Portugal, sensitisation to *Secale cereale* was found to be one of the most prevalent among grass species (58). Similar results were found among children in a French study (54). Likewise, in Hungarian adults with hay fever, cultivated rye sensitisation accounted for 63% when, in general, 84% of these patients were sensitised to non-cultivated ryegrass (59).
In another study (60), nasal provocation testing was used to confirm sensitisation in patients with respiratory allergies to aeroallergens, so that to proceed with immunotherapy. All patients sensitised to rye pollen were also sensitised to grass pollen. In addition to that, all patients with positive nasal provocation tests to rye pollen tested positive also to grass pollen extracts, although the reverse did not apply to all grass allergic patients.
Immunotherapy treatment with an extract containing a grass mixture with or without *Secale cereale* extract was applied to two groups of allergic patients. One to three years after, IgG antibody responses to the major group V grass pollen allergens, including allergen Sec c 5, were found to be cross-reactive up to 92%, indicating that immunotherapy treatment with just one grass species might be sufficient. However, species specific antibodies did also exist (56).
There are several other studies in which patients allergic to grass and/or rye pollen with allergic rhinoconjunctivitis and/or asthma have been treated with specific immunotherapy. Unfortunately, the design of these studies does not allow for specific conclusions concerning the role of rye pollen extract to the immunotherapy outcome (61-63).

Wheat (Triticum spp.)

Triticum species and in particular *Triticum aestivum* are important members of the grass family (64). 1.3-β-glucanase allergen, a member of the pathogenesis-related protein family, is the only known allergen in wheat-pollen as opposed to many food allergens contained in wheat-seed (65).
It should be pointed out that allergens from wheat can mediate pollen allergy, food allergy and baker's asthma, three different entities that must be distinguished from each other. Baker's asthma is an occupational allergy in which sensitisation occurs via inhalation of cereal flours, mainly from wheat but also from rye and barley seeds (66). Five recombinant wheat-seed allergens have been specifically recognised in patients with allergies to wheat seed products, but not in grass pollen allergic patients, suggesting a useful diagnostic method to discriminate wheat-pollen versus wheat-seed-induced forms of allergies (67).
Epidemiologically, sensitisation to cultivated wheat has been found to be one of the most prevalent among multi-sensitised patients to aeroallergens with respiratory allergies (68). Lack of standard recruitment criteria may account for the wide range of sensitisation frequency from 17.7% to 60.8% found in different studies (16, 48).

Corn (Zea mays)

Immunological and biochemical studies showed that Zea m 13 and, to a lesser extent, Zea m 1 are the most prominent maize pollen allergens. Timothy grass pollen extract completely blocked IgE binding to maize pollen allergens, while the opposite occurred partially (38). This could be attributed to the absence of detectable group 2 and 5 allergens to *Zea mays* (37).

Sensitisation to maize pollen was found to be highly prevalent among Turkish (43), South African (69) and Mexican (70) patients with respiratory allergies. In an additional Mexican study (33), sensitisation status was assessed in a cohort of 661 patients. 50 patients (7.6%) with rhinitis and/or asthma had clinically relevant sensitisation to maize pollen. Interestingly, all of them were farmers in corn fields, emphasising the role of maize pollen as an occupational allergen. In addition, 125 of the 661 patients examined were found to be sensitised to maize but with no relevant clinical history (33).

To our knowledge, in the literature there is only one case report of an individual with respiratory allergies, monosensitised to maize pollen extract, without simultaneous sensitisation to wild grass pollen. This patient had a marked improvement in respiratory symptomatology after immunotherapy treatment (71).

Lastly, there is only one study in which maize pollen extract was included in grass pollen immunotherapy in a double blind placebo control manner, but without a sample of patients monosensitised to maize pollen to compare with (72).

Millet (Panicum spp.) and Sorghum (Sorghum bicolor)

Sensitisation to these two cereal member have been assessed rarely. In one study sensitisation to millet pollen ranged from 78.3% to 82.1% (21), while in another sensitisation to sorghum pollen reached approximately 20% (50).

Conclusion

Pollen allergens of the Poaceae family comprise one of the main causes of pollinosis worldwide. Allergens from non-cultivated grasses are almost all the times responsible for respiratory symptoms in grass-sensitised individuals. In these patients, appropriate diagnostic approaches, like skin prick testing and serum specific IgE determination, should be followed. However, aerobiological and environmental characteristics should be taken into account, along with known sensitisation patterns and cross-reactivity data, before final diagnosis is concluded and treatment like immunotherapy is considered. Pollination and aerobiological features of cereals do not include them among the most common causes of clinically relevant pollen sensitisation. Their cross reactivity with the major representative of the non-cultivated grasses, timothy grass, may account for the high percentage of sensitisation levels concluded in various epidemiological studies. However, most studies agree that only in a minority of patients associated with close contact with cereal pollen, allergy should be considered in the differential diagnosis, and should be approached and treated accordingly. More studies are needed in order to evaluate the role specific immunotherapy may have in patients sensitised to both wild and cultivated grass pollens, and to assess the concordance of serum specific IgE levels and skin prick test responses with nasal or conjunctival provocation tests.

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