Wind-pollination and the roles of pollen allergenic proteins

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Summary

Over the past few decades, there has been an explosion of understanding of the molecular nature of major allergens contained within pollens from the most important allergenic plant species. Most major allergens belong to only a few protein families. Protein characteristics, cross-reactivity, structures, and IgE binding epitopes have been determined for several allergens. These efforts have led to significant improvements in specific immunotherapy, yet there has been little discussion about the physiological functions of these proteins. Even with large amounts of available information about allergenic proteins from pollens, the incidence of pollen allergy continuously increases worldwide. The reason for this increase is unclear and is most likely due to a combination of factors. One important culprit might be a change in the pollen itself. Knowledge about pollen biology and how pollen is changing as a result of more extreme environmental conditions might improve our understanding of the disease. This review focuses on the characteristics of plants producing allergenic pollens that are relevant to pollen allergy, including the phylogenetic relationships, pollen dispersal distances, amounts of pollen produced, amounts of protein in each type of pollen, and how allergenic proteins are released from pollens. In addition, the physiological roles of major allergenic protein families will be discussed to help us understand why some of these proteins become allergens and why GMO plants with hypoallergenic pollens may not be successful. ***(Asian Pac J Allergy Immunol 2013;31:261-70)***

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Introduction

Pollen is a highly specialized part of the male reproductive system from gymnosperms (naked seed plants) and angiosperms (flowering plants). It plays a crucial role in delivering sperm nuclei to the female reproductive parts, thereby achieving sexual reproduction. This pollination process can be accomplished by several means, the simplest of which is wind pollination. In wind pollination, pollens are released into the air. Unfortunately, the immune system of certain individuals produces IgE that can recognize these airborne pollens as foreign and harmful substances and mounts the immune response, leading to allergic symptoms. Even though pollen allergy is usually not life-threatening, it can severely affect the quality of life of patients. Unlike food allergens, airborne pollen can hardly be avoided because of its extremely small size and high prevalence in the air. For those with significant symptoms, medication must be taken daily during the blooming season, resulting in drowsiness and reduced productivity. This review will discuss some features of wind-distributed pollens that might be relevant to their allergenicity. Understanding pollen biology might shed light on improving prevention and therapy for pollen allergy in the future.

Pollen production and function

Pollen is a male gametophyte of seed plants produced from meiosis of microspore mother cells in the anther. Each of the four daughter cells then undergoes asymmetric mitosis to produce a larger vegetative cell and a smaller generative cell. The generative cell containing a condensed nucleus and little cytoplasm is completely surrounded by the vegetative cell. As the pollen matures, RNA and proteins needed for the early stages of pollen germination are deposited in the cytoplasm of the vegetative cell. The pollen wall is composed of a complex polymer called sporopollenin. The outer layer of the pollen wall, or exine, has a specific structure that is highly useful for pollen identification. Pollen is then coated with lipids, phenolic compounds and several proteins.[1,2] The pollen coat composition is critical for species recognition and also for protection against environmental damage.[2] After pollen maturation is completed, anther dehiscence occurs to facilitate pollen dissociation and dispersal. To increase genetic variability, several plant species have evolved a complex mechanism that prevents self-fertilization called self-incompatibility (SI). This requires pollen traveling beyond the plant from which it was produced. To perform this role, pollen must be able to withstand the harsh environmental conditions during the voyage.

Mature pollen released from the anther must somehow find a way to land on the tip of female reproductive organ, the stigma. The pollen then proceeds through five steps to achieve fertilization: germination, stigma invasion, growth in the transmitting tract, growth in the ovarial cavity, and gamete interaction.[3] Essentially, the vegetative cell undergoes polar expansion, making a pollen tube that delivers the male gamete through transmitting tract until it reaches the female gamete located in the ovule. The nucleus of the generative cell divides to generate two nuclei. One nucleus will fertilize the egg, forming zygote. The other will fuse with the center nucleus, forming the endosperm, which will act as food source for the growing embryo.[4,5] In short, pollen acts as a vessel for the delivery of male gamete to fertilize the female gamete. During this entire process, pollen must undergo rapid changes. Only the first pollen tube to reach the ovule has an opportunity to release the sperm nucleus for fertilization. In fact, pollen tube growth is one of the most dramatic changes observed in plant cells. Because of the rapid growth of the plant cell wall, this process requires large-scale cell wall modification. New cell wall materials must be delivered to the growing site, generally through vesicular trafficking along the cytoskeleton. Furthermore, cell shape changes rapidly, requiring the continuous cytoskeleton rearrangement. Pollen tube growth is a directional growth down the transmitting tract toward the ovary. This implies that a certain kind of signal and a supporting network of molecules for signaling are required. In pollen germination and pollen tube growth, one of the most significant signaling mechanisms is through calcium signaling. Diagrams of typical plant reproductive organs are shown in Figure 1.

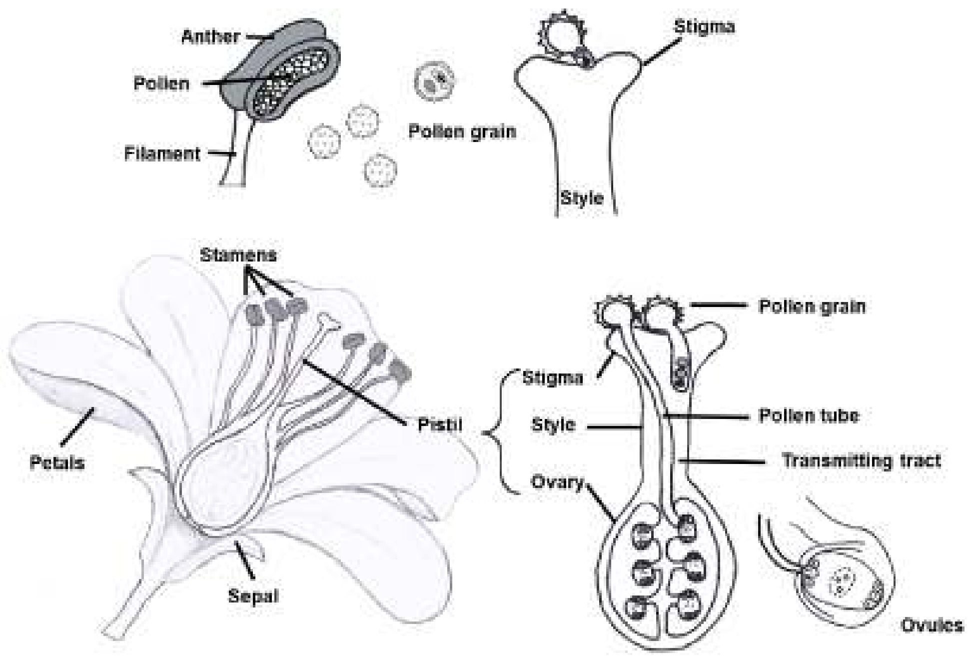

Figure 1. Male and female reproductive organs of plants. Pollens are two-cell male gametophytes produced by the anther. After dispersal, pollens landing on the right stigma will germinate and grow pollen tubes down the transmitting tract to reach the ovary. One nucleus fuses with the egg nucleus to form a zygote; the other fuses with the center nucleus, forming endosperm.

Features of wind pollination and implications on pollen allergy

Most allergenic pollens are produced from abundantly distributed wind-pollinated plants. Wind pollination is among the most common pollination methods. Wind-pollinated plants constitute approximately 10-18% of all flowering plants, including some deciduous trees, several weed species, most grasses, and almost all conifers.[6,7] These species can be recognized by their small, unscented flowers with dull color. Although wind-pollination can be considered the simplest method, several characteristics have been developed through evolution to enhance the chance of fertilization. Notable examples are the small dry pollens, some with wing-like structure aiding their flight, and thin filaments supporting anthers to catch the slightest air movement for pollen dispersal. Nonetheless, the best way to ensure that some pollen will land on the right stigma is to produce a large amount of pollen. Because there are many plant species producing enormous quantities of pollen to achieve sexual reproduction, there is plenty of airborne pollen during the blooming season.

There are several features of wind pollination that have significant implications for pollen allergy, some of which are discussed below.

1) Wind-pollinated pollens are usually small and can be dispersed over a long distance. Pollens that can stay airborne longer can be distributed farther and have higher chance of successful fertilization. Thus, most wind-pollinated species have small pollens with diameters ranging around 20-40 μm. Long-distance transport of allergenic pollen has been previously reported.[8] In addition, wind-pollinated pollens can have special structures to improve the aerodynamics and increase dispersal distance, such as the air-filled sacs found in pine pollens.[9] Pine and spruce pollens had been shown to travel as far as 3,000 km.[10] In one study, viable loblolly pine (*Pinus taeda*) pollens could be found at 610 meters above ground, and at least 41 km from the source.[11] Because these pollens are viable, it can be assumed that the proteins contained within, including allergenic proteins, remain intact. The dispersion range of several wind-pollinated species has also been studied in response to concerns about genetically modified crops such as bentgrass (40-55km).[12] The fact that wind-pollinated pollens can travel such a long distance has grave consequences for pollen allergy sufferers, because it may be nearly impossible to avoid certain types of pollens unless they move to a different city. It is important to note that there is no evidence that the shape of pollen is related to allergenicity. Hence, the spiny (echinate) pollens of plants in the sunflower family (Asteraceae) are not more allergenic than the round pollens of grasses.

2) Large amount of pollen must be released to increase the efficiency of fertilization. Flowers of wind-pollinated species often lack large petals, colors, and fragrance. To maximize pollination success and seed production, wind-pollinated species produce numerous, small, flowers with a single ovule per flower.[13] These flowers specialize in producing large amounts of pollens. Each maize plant, for example, can produce approximately 0.2-2 million pollen grains per day.[14] Moreover, this type of flower usually blooms synchronously.[15] As a result, the concentration of pollen in a corn field can be as high as 945 grains/ m^3.[14] Similarly, a rice field can produce up to 386 grains/m^3.[16] Pollen-allergic patients are recommended to avoid exposure to agricultural fields during the blooming season, especially those containing crops in the grass family (Poaceae), such as rice, wheat, maize (corn), barley, rye, sugar cane, sorghum, millet, oats, and grasses for animal feed. Certain lawn grasses, such as Bermuda grass (*Cynodon dactylon* (L.) Pers.) and Manila grass (*Zoysia matrella* (L.) Merr.), can produce tiny flowers all year round in the tropics. Therefore, the lawn must be cut frequently to prevent blooming. When travelling to unfamiliar territory, patients should avoid trees or weeds producing numerous, small, dull-color flowers with no obvious petals.

3) Wind-pollinated pollens are dry and require hydration to stimulate germination. Anther dehiscence is a carefully orchestrated process involving jasmonic acid and auxin hormones, several enzymes, and programmed cell death.[17] The water content in mature pollen grains is only about 10-30%.[18] Upon landing on the stigma of the female reproductive organ, pollen hydration occurs in a controlled manner through aquaporin.[19] However, if dry pollen is immediately exposed to bulk water, the increased osmotic potential can lead to loss of membrane integrity and pollen damage, spilling out the cellular content.[20] Therefore, proteins contained within pollen can be released by triggers such as high humidity, rain, or air pollution. Allergen release also depends on pH and temperature. For example, the optimal condition for protein release from birch, grass, and pine pollens is 37°C and pH

9.0.[21] Once released, pollen protein particulates suspended in the air are still able to cause allergy.[22] These particulates are much smaller than whole pollen and might be able to travel longer distances. It also suggests that pollens that can be disrupted easily, so pollens with a thin wall might cause more allergenicity. This is the case for grass pollens (unpublished data). In fact, in aeroallergen surveys, grass pollens are rarely found to be in the original round shape with one aperture. Rather, they are often folded up or damaged. This type of damage can be enhanced by air pollutants.[21-23] Therefore, an aeroallergen survey by pollen count might underestimate the actual amount of allergen in the air. It is important to note that although most allergenic proteins studied so far are located inside the pollen, recent studies showed that there are several proteins produced by the tapetum and deposited on the pollen coat.[5,24,25] Some of these proteins have IgE binding activity.[25]

4) It has been estimated that about 10-18% of flowering plants are wind-pollinated.[6,7] Wind-pollination was previously assumed to be a rudimentary and wasteful mode of pollination. This is partly because wind-pollination is common among gymnosperms such as pines, fir, cypress, cedars, and cycads. Even Charles Darwin remarked on the question of why wind-pollination still exists in the more recently evolved flowering plants (angiosperms). However, more recent studies have shown that wind pollination is not less effective than animal-assisted pollination.[7] Among angiosperms, wind-pollination arose 65 times from animal-pollinated ancestors.[7] This is usually in response to pollinator limitation or climate change. Wind-pollination is more common in the temperate regions than in the tropics because there is less moisture and precipitation, allowing pollens to disperse more efficiently in the wind.[13] Another reason is that temperate regions have shorter and more well-defined pollinating seasons and thus the concentration of airborne pollen is higher. Moreover, successful wind-pollination depends on wind velocity, which is significantly higher in alpine regions compared to forests.[13] This means that pollen allergy tends to be much more prevalent in these regions. In addition, because several wind-pollinated species evolved from insect-pollinated ancestors, it appears that wind-pollinated plants are not more closely related to each other than to animal-pollinated plants. This has implications in terms of pollen allergy. Firstly, based on the updated angiosperm phylogeny group classification (APGIII),[26] plant species producing allergenic pollens are not necessarily closely related. For example, the olive tree (*Olea europaea* L.) and the silver birch (*Betula pendula* Roth) are both highly allergenic, yet belong to different orders (Lamiales and Fagales, respectively). On the other hand, sage, mint and lavender are all members of the Lamiales order and are not significant sources of allergenic pollens. Therefore, one cannot simply use phylogenetic tree of flowering plants to predict allergenicity of their pollens. Secondly, certain plant orders or families may be sufficiently different from others that allergens are restricted to only a few closely-related species. For example, Bet v 1-related proteins are mostly found in pollens from plants in the Fagales order, including birch, beech, oak, hazel, alder and chestnut.[27] Likewise, all grasses belong to the Poaceae family and thus they produce similar allergens that can elicit extensive cross-reactivity.[28-30]

5) Climate change has a significant impact on airborne pollen because it increases the growth rate, the amount of pollen from each plant, the amount of protein in each pollen, and the pollen season.[31,32] In a 27-year study of airborne pollen in Italy, it was shown that the total pollen of some species investigated had increased approximately 25%.[33] The patients sensitized to pollen increased significantly while those sensitized to house dust mite remained constant[33] The impact of climate change on aeroallergen has been extensively reviewed previously.[34-41]

6) Pollen contains a large amount of proteins (~25% of grass pollen and ~30% of olive pollen)[42] Because pollen tube growth is under fierce competition, pollen must be ready to germinate. This means that several proteins, rRNA, and mRNA required for germination and initial growth have to be stored within the pollen prior to desiccation and pollen release.[20] Recent studies of pollen transcriptomes and proteomes showed that there are more genes and proteins in pollen than previously believed.[43-45] One study showed that 3,945 genes, or 17.5% of genes represented on an ATH1 microarray, are expressed in the mature pollen of Arabidopsis.[46] Another study estimated that over 7,235 transcripts could be found in the same tissue.[45] More than 60% of Arabidopsis genes are expressed during pollen development and about 10% of genes are specific to this process.[45] As for proteomic studies, shotgun sequencing showed that ~3,500

proteins are present in Arabidopsis pollen.[43] In tomato and tobacco, the number of proteins found in pollens is much higher than those found in leaves.[44,47] Transcripts and proteins related to cell wall modification, vesicle trafficking, and defense are present in high amount during pollen desiccation.[44,46,47] This is significant because several allergenic proteins belong to these groups of proteins. Even though it is difficult to determine which proteins are the most abundant in pollen, it is clear that the allergenic components are abundant and are required for pollen germination and growth. On the other hand, it is also true that there are many other abundant proteins which have never been shown to be allergenic. Therefore, it can be said that an allergenic protein not only must be present abundantly but also must have other intrinsic properties contributing to its allergenicity.

7) Pollen is subject to extremely high competition. It is often found that several pollens are attached to the same stigma and multiple tubes grow into the transmitting tract. However, only the first pollen tube to reach the ovule is allowed to release sperm nuclei for fertilization. Once gamete transfer occurs, all other pollen tubes are prevented from entering the ovule.[49] Therefore, the faster a pollen tube grows, the higher its reproductive success. It is unsurprising that the pollen tube is the fastest growing plant cell at the rate of more than 10,000 microns per hour.[50] For comparison, the average diameter of wind-pollinated pollens is between 20-40 microns. Even a slight defect in this process will cause the loss of competitiveness. This has implications in the field of hypoallergenic pollen engineering. Because pollen allergens are often proteins with significant functions, altering these proteins might compromise their functions. Few studies have shown the effects of disrupting genes encoding pollen allergens e.g. Lol p 5 of rye grass and EXPB1 of maize.[51,52] In both studies, there was no obvious detrimental effect on pollen viability or growth. However, pollen with the *expb1* mutation had significantly reduced competitiveness because the pollen tube grew significantly more slowly *in vivo* compared to the *EXPB1* wild-type pollen tube.[52] Reduction of competitiveness has two critical consequences. First, in the presence of wild-type pollens, the engineered hypoallergenic pollen with slightly slower growth has little chance of reproductive success and is unlikely to pass on the hypoallergenicity to the next generation. Without continuous attempts to maintain this variety, it is most likely to be eliminated from the gene pool quickly. Secondly, in the case of grain crops such as wheat, rye, rice, and maize, each successful fertilization event leads to the production of one grain. Because the highest grain yield is expected, a cultivar producing. Therefore, the cultivar producing hypoallergenic pollen with reduced fertilization rate is unlikely to be preferred by farmers. However, for plants that can be propagated asexually such as landscape trees, genetic engineering for hypoallergenic pollen may significantly reduce the amount of airborne pollen.

Major allergens and their physiological roles in pollen

Architectural and structural features of allergenic proteins seem to contribute significantly to their allergenicity. One strong indication is that pollen allergens from almost all plant species belong to only a few (29 out of 7,868) protein families.[53,54] Keep in mind that not all members of the family are allergenic, possibly due to the fact that humans are not exposed to each member of the family to the same level. Thus, allergenicity of a given protein is not only dependent on the amino acid sequence or structure of the protein, but also related to the localization and amount of the protein. Generally, cells keep a tight control over the concentration and localization of proteins. The fact that allergenic proteins are present in high amount indicates that these proteins have physiological roles required in pollen. Based on data from Allfam (http://www.meduniwien.ac.at/allergens/allfam/), the top 5 families of pollen allergens are profilin, expansins, prolamin, EF-hand domain containing proteins, and Bet v 1-related proteins.[55] These seemingly arbitrary families of proteins indeed play significant roles in pollen germination and growth. A diagram illustrating functions of top allergenic proteins is shown in Figure 2.

\- *Stigma penetration requires cell wall loosening activity: expansin*

As previously described, the function of pollen is to deliver sperm nuclei down the transmitting tract into the ovary. This task requires that the pollen tube inserts itself in between cells of the stigma and transmitting tracts. Mature plant cells are normally surrounded by a cell wall. Adjoining cell walls are cemented by middle lamellae composed of pectin. This barrier is extremely difficult to breach. Pollen achieves this feat without permanent damage to cell

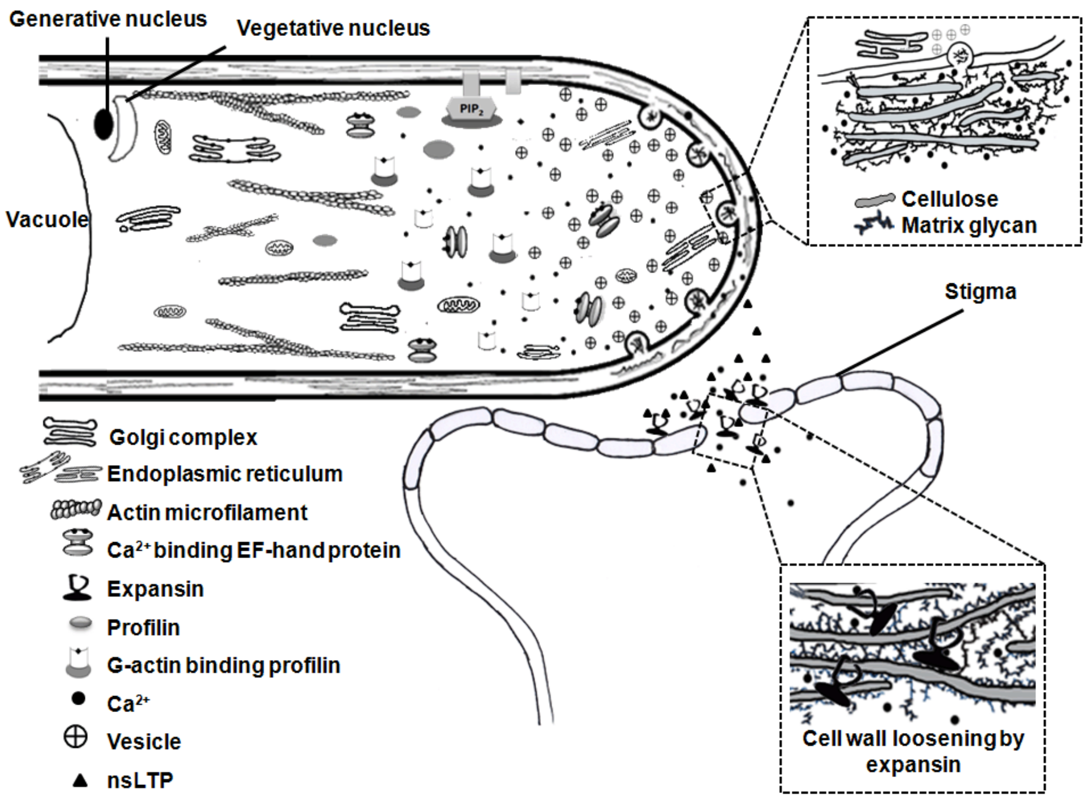

Figure 2. Functions of top allergenic proteins in pollen growth. Expansins perform cell wall-loosening function to allow penetration of the stigma. Calcium signaling pathways required for pollen tube growth are mediated by calcium binding proteins such as EF-hand proteins. Polar growth of pollen tube requires massive cycling of vesicles along actin microfilaments. Profilin binding with actin modulates actin microfilament polymerization. The secreted nsLTPs may function in pollen tube guidance down the transmitting tract in an autocrine-like manner.

walls by secreting a cell-wall loosening protein called expansin.

Expansin is a glycoprotein consisting of 250-275 amino acids. Even though expansins are found in both dicots and monocots, allergenic expansins have only been identified from grass pollens. These grass pollen expansins are collectively referred to as group I grass pollen allergens. Expansin is a major allergen of grass pollen, with IgE reactivity in about 90-95% of grass pollen allergic patients.[56] Because they are significantly different from the vegetative isoforms (alpha-expansins), grass pollen expansins are grouped into the beta-expansin family.[57] Examples of characterized allergens in this family are Cyn d 1, Lol p 1, Phl p 1, Ory s 1, Dac g 1, Pas n 1, and Zea m 1.[55] Cross-reactivity among expansins from different grass species is high.[56,58] Four isoforms of beta-expansin can be found in maize pollen.[59] Expansins from maize pollen were shown to localize mostly in the cytoplasm.[60] However, signal peptide analysis suggests that it is likely to be secreted by pollen during pollen growth.[57] The known function of expansin is to catalyze cell wall loosening during cell growth and other developmental processes.[57,61] Interestingly, expansin does not possess any proteolytic activity.[62] Instead, the protein can alter non-covalent bonding of glycans on the surface of cellulose.[63] This action allows slippage of cellulose microfibrils, resulting in cell wall loosening without permanent damage.[63] A study of maize pollen showed that a beta-expansin mutation led to abnormal pollen separation and penetration of the stigma.[64] Beta-expansin, therefore, might function to modify tightly appressed cell walls of the stigma and the transmitting tract, allowing the pollen tube to grow through and between the cells of these maternal tissues[57] Alpha-expansin functions on xyloglucan, which is more abundant in the type I cell wall of dicots.[63,65] The significantly different beta-expansin of grasses prefers other glycans, such as xylan, which are present in higher amount in the type II cell wall of grasses and sedges.[63,65] This is likely to be the reason why allergenic expansins have only been identified from grasses.

- *Pollen tube growth requires rapid change of cytoskeleton: profiling*

Profilin is an actin-binding protein conserved in all eukaryotes. Interestingly, only plant profilins have been shown to be allergenic. Allergenic profilins have been identified from pollens of more than 20 plant species, including Cyn d 12 from Bermuda grass, Bet v 2 from Birch, Ole e 2 from Olive tree, Phl p 12 from Timothy grass, and Zea m 12 from corn.[55] Each plant species can have several profilins in its genome. Profilins can be divided into two groups based on expression pattern: constitutive or vegetative profilins and reproductive profilins.[66] For example, there are five isovarients of profilins in *Arabidopsis thaliana*, with *PRF1*, *PRF2*, and *PRF3* expressing in vegetative tissues and *PRF4* and *PRF5* expressing specifically in pollen.[66] Because profilins are expressed in both vegetative and reproductive tissues, they often cause pollen-food syndrome.[67] High polymorphism of profilins suggest that they are multifunctional, perhaps with different partners.[68] Compared with other organisms, plants have a larger variety of actin and actin binding proteins.[69] Other than binding with actin, profilins can also interact with PtdIns(4,5)P_2, poly-L-proline (PLP) and proline-rich proteins, and several other proteins.[70] Biochemically, there are two classes of profilins with different binding preferences. Because of these properties, profilins can cause both actin polymerization and depolymerization.[70] The concentration of profilin in hydrated, ungerminated poppy pollen was found to be about 234 uM, which is similar to the concentration of actin monomer, suggesting a 1:1 binding ratio.[68] Therefore, profilin regulates actin polymerization by sequestering or releasing actin monomer in response to cytoplasmic Ca^{2+} concentration during pollen growth.[70] In SI, an increased Ca^{2+} concentration can lead to rapid actin depolymerization through the action of profilins.[71] While changes in Ca^{2+} concentration during pollen growth can regulate profilin activity and the state of actin filaments, profilin activity can also affect the Ca^{2+} level. Profilin can bind to PtdIns(4,5)P_2 and prevent its hydrolysis by PLC. PLC hydrolysis of PtdIns(4,5)P_2 produces IP3, which is a secondary messenger that can trigger cytoplasmic Ca^{2+} release.[70]

- *Pollen tube growth is guided by calcium signaling: EF-hand domain proteins*

The growth of pollen tubes is dependent on calcium. In fact, pollen has been an excellent model for calcium signaling studies in plants. The role of calcium and downstream signaling during pollen growth has been addressed in several recent reviews.[72-74] The Ca^{2+} concentration is especially high at the growing tip. Oscillations of Ca^{2+} levels lead to a signaling cascade mediated by Ca^{2+} binding proteins. A large number of Ca^{2+} binding proteins contain a characteristic helix-loop-helix structure called the EF-hand domain. Because calcium signaling is used ubiquitously for plant growth and development, plant genomes contain a large family of EF-hand containing proteins. The Arabidopsis genome has up to 250 EF-hand containing proteins, constituting about 10% of all its genes.[75] For comparison, only 2% of genes in the human genome contain EF-hand motifs.[75] Each EF-hand containing protein may consist of multiple EF-hand domains, ranging from 1-6 in plants.[75] The number of EF-hand motifs is used for classification of the proteins in this family.

Allergenic EF-hand proteins from pollens are mostly small proteins (9 kD) containing two EF-hand motifs, also known as polcalcins.[55] Pollen allergens in this family include Aln g 4, Amb a 9 & 10, Art v 5, Bet v 3 & 4, Bra n 4 & 7, Bra r 4 & 7, Che a 3, Cup a 4, Cyn d 7, Jun o 4, Ole e 3 & 8, Par j 4, Phl p 7, and Sur v 3[55] Calcium binding has been shown to significantly affect protein conformation and IgE binding of Phl p 7, Bet v 4, and Ole e 8.[15,50,76,77] Another study showed that allergens with 2, 3, and 4 EF-hand domains shared similarities and can be cross-reactive.[78] Calcium-induced conformational change and the effects on IgE binding properties can be examined using SDS-PAGE, circular dichorism spectroscopy and ELISA.[79] Even though the exact function of allergenic EF-hand proteins in pollen is not known, the structure of Phl p 7 suggests that they are more likely to function in ligand binding rather than as a calcium buffer.[15]

- *Pollen tube must be guided down the transmitting tract: nsLTPs and Ole e 1-related proteins*

nsLTPs or non-specific lipid transfer proteins are small proteins with diverse functions. nsLTPs form a large and complex protein family with distinct expression patterns and are considered members of the prolamin superfamily.[55] Other allergens belonging to this superfamily are seed storage proteins and amylase/protease inhibitors found in fruits, nuts, and grains. There is a significant cross-reactivity among members of the prolamin superfamily, causing the pollen-fruit syndrome. Because *nsLTP* expression level is low in pollen, it is believed that primary sensitization to nsLTPs usually occurs by the oral route.[80]

nsLTPs are characterized by four alpha helices cross-linked with four disulfide bridges using eight cysteines.[81] Because all nsLTPs have an hydrophobic internal cavity, they were believed to function in lipid transfer. However, nsLTPs bind non-specifically to lipids and are also secreted into the extracellular matrix, suggesting that they have roles outside the plasma membrane.[82] In fact, no *in vivo* LTP-lipid complex with biological significance has been isolated.[82] More recent studies suggest that nsLTPs play significant roles in defense, cell wall loosening activity, and/or signaling.[80-83] Several ns*LTPs* have strong expression in the reproductive tissues.[83] The most well-known example is the lily SCA protein, which is secreted by the transmitting tract and plays a significant role in forming an adhesive matrix for pollen tube guidance.[82] *LTP5* was shown to have low level of expression in the pollen and transmitting tract of Arabidopsis. Nonetheless, overexpression or mutation of this gene results in a swollen pollen tip and growth arrest, suggesting its role is critical in establishing polarity in an autocrine-like manner.[84]

Interestingly, LAT52, a highly expressed protein in mature pollen of tomato, has been shown to perform a similar function.[85] It is secreted by the pollen tube and binds to its receptor, LePRK2, which is located at the plasma membrane of the pollen tube itself.[85] As with nsLTPs, LAT52 is also a cysteine-rich extracellular protein. LAT52 does not belong to the nsLTP family; instead it is highly similar to Ole e 1, a major allergen from olive tree. Members of the Ole e 1-related protein family are known allergens of trees in Oleaceae family (ash, lilac, privet), grasses (group 11 grass pollen allergens), and weeds (plantain and lamb's quarters).[55] Although the biological functions of these proteins have not been proven, it is likely that they play significant roles in pollen tube guidance similar to LAT52.

- *Pollen must defend itself from harsh conditions: pathogenesis-related (PR) proteins*

During dispersal, pollens are exposed to harsh conditions, such as UV radiation and heat. After a day of exposure, UV-A and UV-B can cause 10% reduction of pine pollen viability and overall viability is reduced to 50% by typical outdoor conditions.[48] In order to protect the highly valuable genetic information contained within, pollens are loaded with defense proteins. Importantly, several groups of pathogenesis-related (PR) proteins had been classified as allergens from pollens, fruits, and latex.[86] These proteins share similarities in that they are small proteins, stable at low pH, and resistant to proteolysis.[87] The PR protein families important for pollen allergy are PR-10 (Bet v 1-related proteins) and PR-14 (LTPs).

As mentioned above, nsLTPs can function as defense proteins. Some *LTP* genes are known as PR-14 genes and are highly resistant to heat and digestive enzymes.[82] The arabidopsis genome contains about 100 *LTP* genes. *LTP2*, *LTP5*, and *LTP6* have been shown to be inducible by abiotic (drought and salt) stresses.[83] The DIR1 and AZI1 proteins in Arabidopsis are involved in pathogen defense responses, while Ace-AMP1 from onions and LTP4 from barley show antimicrobial activity.[83]

Bet v 1-related proteins are intracellular proteins with unknown enzymatic activity.[87] Bet v 1, the major birch pollen allergen, elicits IgE reactivity in over 90% of individuals allergic to birch.[87] Furthermore, Bet v 1 causes significant cross-reactivity with other allergenic members of PR-10 family, especially those present in food, such as Mal d 1 from apple, Pru av 1 from sweet cherry, Pru ar 1 from apricot, Api g 1 from celery, and Cor a 1 from hazelnut.[87]

Because PR proteins can be induced by development, abiotic stresses, and pathogens, the level of allergenic proteins present in airborne pollens may differ significantly. For example, the cedar pollen allergen Jun a 3 has variable expression across years and geographical areas.[86] With climate change and more extreme environmental conditions, the amounts of PR proteins is likely to increase, which may partially account for the continuous increase in allergy incidences worldwide.

Conclusion

Pollen plays a special role in the life cycle of angiosperms and flowering plants. To increase the chance of successful pollination, wind-pollinated plants have evolved special features, such as the numerous light-weight pollens that are synchronously released and can be dispersed over a long distance. These features have a significant impact on the field of pollen allergy. At the molecular level, allergenic proteins have critical functions in the highly competitive process of pollen germination and pollen tube growth. In this process, expansins catalyze temporary cell wall loosening. Profilins are involved in the rapid modification of the actin filaments, while EF-hand proteins are involved in Ca^{2+}-mediated signaling. Ole e 1-related proteins and nsLTPs may play a role in pollen tube guidance. The variable expression levels of PR proteins suggests that environmental factors have a significant impact on immunoreactivity and allergic symptoms.

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