DECISION SUPPORT DOCUMENT[1]

Maize and *Bacillus thuringiensis Cry* protein allergenicity

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Abstract

The aim of this document is to summarise and interpret the current literature regarding maize allergenicity and the possible contribution of *Bacillus thuringiensis* (Bt) Cry proteins to allergenicity in transgenic maize lines. We provide a brief overview of allergenicity and discuss the molecular mechanisms that could introduce new proteinaceous allergens in plants. Maize allergenicity is discussed in terms of the possible routes of exposure and the known maize allergens are divided into food and pollen allergens. Similarly, general Bt and specifically Cry protein allergenicity is discussed based on the available literature. We conclude that an allergic reaction to a particular Bt maize cultivar would more likely be caused by a known maize allergen than the Cry protein, although this does not exclude the possibility of a chance mutation to the Cry protein (or any other maize protein) that could somehow cause it to be allergenic.

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Introduction

Allergies are immunological disorders, characterised by a hypersensitive reaction of the immune system to normally harmless substances, often proteins, known as allergens. Production of immunoglobulin E (IgE) antibodies against the allergen leads to the excessive activation of certain white blood cells that result in an excessive inflammatory response in an allergic individual. Typical symptoms include allergic conjunctivitis (red eyes), allergic rhinitis (a runny nose) and contact dermatitis (skin inflammation/rash). Common allergic reactions include hay fever, food anaphylaxis, eczema and allergic asthma and can be caused by pollen, mould spores, mites, animal hair, food, drugs, the venom of stinging insects, etc. These reactions occur in atopic individuals who are genetically predisposed to a specific allergy and who have been previously sensitised by an amount of the allergen sufficient to induce an immune response. Allergies can be diagnosed by checking for skin responses to suspected allergens (skin pricks) or by analysing the blood for the presence and levels of allergen-specific IgE (Kay, 2000; Sicherer, 2000).

During recent years there has been a marked increase in the prevalence of atopic disease among people with a “Westernised life-style”. Moreover, IgE-mediated allergic reactions affect 10 to 25 % of the population in developed countries (Mekori, 1996; von Mutius et al., 1998). The incidence of food allergies specifically ranges from 1 to 2 % in adults and 6 to 8 % in children. A small number of food types (6 to 8) are responsible for the vast majority (>90%) of significant food-induced allergic reactions. In adults the most common allergies are to shellfish, peanut, tree nuts, fish and more recently sesame seeds. In general, the most common causal foods in children are cow’s milk, egg, peanut, wheat (gluten), soy, tree nuts, fish and shellfish (Anderson, 1996; Sampson, 1997; Ladics *et al*., 2003; Batista *et al*., 2005).

General pollen allergies affect about 10% of the population in industrialised countries (Lehrer *et al*., 1999). Patients with pollen allergies also frequently experience allergic reactions to ingested food from the same plant because conserved allergens are often expressed in different tissues in the plant. For the same reason the cross-reactivity of IgE antibodies can induce allergic symptoms to related or even unrelated plant species, e.g. an individual that has an allergic reaction to a particular maize pollen protein could have been sensitised by the pollen of a wild grass species (Valenta and Kraft, 1996).

In theory, any protein could potentially trigger an allergic reaction, but it does so only when certain very specific conditions are met. The two most important determinants of allergenicity are identity and exposure. (i) Identity: The surface of an allergenic protein must present a particular epitope that is able to bind to an IgE antibody produced by a susceptible individual’s immune system. This antigen-antibody interaction is highly specific and depends on the genetic predisposition of the individual. (ii) Exposure: To induce an immune response the potential allergen must get an opportunity to interact with an individual’s immune system. Any conditions that increase the likelihood and magnitude of this interaction would therefore contribute to the allergic potential of a protein. This does not only refer to physical exposure through direct contact, ingestion or inhalation (“quantitative exposure”), but also to molecular mechanisms that would contribute to the stability and accessibility of the potential allergen (“qualitative exposure”). Direct or indirect (also natural and induced) genetic mutations could therefore potentially induce allergenicity by increasing the concentrations of a known allergen, enhancing the binding to IgE antibodies (e.g. improved presentation on the protein surface or epitope structure), introduction of glycosylation sites or by increasing the stability of the protein (e.g. reduced protease susceptibility).

Maize allergenicity

Maize induced allergic reactions can be grouped into (i) food allergy due to ingestion (Pastorello et al., 2009), (ii) pollen-induced respiratory allergy (Gonzalo-Garijo et al., 2004) and (iii) occupational asthma or dermatitis due to inhalation or handling of maize flour (Weichel et al., 2006). The prevalence of maize allergy is not well documented but it is thought to be fairly uncommon and has for that reason not been studied extensively (Scibilia et al., 2008; Venter et al., 2008).

Food allergy: Maize food allergy is caused by proteins in the kernels. The website of the International Union of Immunological Societies (IUIS) lists only nonspecific lipid-transfer protein (LTP; Zea m 14) and thioredoxin (Zea m 25) as maize food allergens (http://www.allergen.org). LTP, which is also an important allergen in fruits, nuts, various vegetables and other cereals, has been identified as the major maize food allergen (Pastorello et al., 2000). The nine kDa LTP is extremely stable, it is resistant to heating (it is the only maize allergen that maintains its IgE-binding capacity after heat treatment at 100ºC for 160min) and gastro-intestinal digestion, which makes it a strong food allergen. Trypsin inhibitor (Pastorello *et al*., 2000) and thioredoxin (Weichel et al., 2006) were shown to be minor maize food allergens. Recently several other proteins were also identified as maize food allergens in patients with positive food challenge tests or with a history of maize induce anaphylaxis (Pastorello *et al*., 2009; refer to Table 1 for more details).

Table 1. Proteinaceous maize food allergens.

Protein	Mw (kDa)	Allergen Name	Accession Nr
Azs 22-12 (22kDa alpha-zein 5)	25	Unassigned	Q9SYT3
Endochitinase A precursor	30	Unassigned	P29022
Endochitinase A precursor (seed chitinase A)	30	Unassigned	P29022
Endochitinase B precursor (seed chitinase B)	30	Unassigned	P29023
γ-zeathionin	5	Unassigned	P81009
γ-zeathionin 2	15	Unassigned	P81009
Globulin-2 precursor	50	Unassigned	S15675
Nonspecific lipid-transfer protein	9	Zea m 14	P19656
Subtilisin/chymotrypsin inhibitor	7	Unassigned	S61830
Thioredoxin h1	14	Zea m 25	CAI64400
Trypsin/factor XIIA inhibitor	16	Unassigned	P01088
zein-alpha precursor	26	Unassigned	P06678
Zein-α	25	Unassigned	P24449
Zein-β	20	Unassigned	P06673

Pollen allergy: Mature maize pollen contains more than 1000 different compounds and releases approximately nine different proteins in a watery medium (Suen et al., 2003). Pollen allergens are classified into 10 different allergen groups based on their biochemical structure and immunological reactivity (Andersson and Lidholm, 2003). Zea m 1, i.e. a group 1 allergen, is the most important maize pollen allergen and has been identified as four different isoforms of β-expansin (Li et al., 2003). In addition, profilin (Zea m 12, Weichel et al., 2006), Zea m 13 (unknown function; Heiss et al., 1996) and possibly Zea m 3 (unknown function; Petersen et al., 2006) have been identified as maize pollen allergens (Table 2). Immunological-based proteomic analyses of maize pollen could not identify any novel, maize-specific allergens but confirmed cross-reactivity with known grass allergens (Petersen et al., 2006).

Table 2. Proteinaceous maize pollen allergens.

Protein	Mw (kDa)	Allergen Name	Accession Nr
β-expansin	35	Zea m 1	AY197353
Profilin	14	Zea m 12	AAB86960
Unknown function	55	Zea m 13	X57627
Unknown function	12	Zea m 3	AY331720

Occupational asthma or dermatitis: The IUIS lists only β-expansin and profilin as maize pollen allergens (http://www.allergen.org) while the Food Allergy Research and Resource Program (FARRP; University of Nebraska) also lists Zea m 13 and 25 (thioredoxin h1) as maize aeroallergens (http://www.allergenonline.org). In addition, the maize food allergens thioredoxin h1 (Zea m 25) and trypsin/factor XIIA inhibitor have been identified as contributing allergens to Baker's asthma, which affects a significant proportion of workers in the food industry who are exposed to the inhalation of wheat or maize flour dust (Weichel et al., 2006). Under these specific circumstances maize food allergens could therefore also act as aeroallergens (with primary exposure through inhalation) in even if they are only expressed in maize kernels.

Bt Cry protein allergenicity

Bt Cry proteins are naturally occurring insecticides which have been used in spray-on formulations since the 1920s (Glazer and Nikaido, 2007). Using whole Bt spore extracts, i.e. a mixture of many of the organism's proteins, Bernstein et al. (1999) demonstrated skin sensitisation in 2 of 123 farm workers with previous respiratory exposure to Bt sprays, but all subsequent studies on purified Cry proteins suggests a lack of allergenic concern for the Cry proteins (Ladics et al., 2006; Lemaux, 2008). Searches in the international allergen databases listed in Table 3 yielded no references to any general "*Bacillus thuringiensis*" or specifically "Bt Cry protein/endotoxin" allergens. The only allergen database with references to Bt Cry proteins is *Allergome*, a comprehensive information platform that lists all allergen related research. Cry1Ab, Cry1Fa and Cry9c are listed as "negative" (non-allergenic) in non-functional IgE tests and "always negative" in the epidemiological literature. Cry9c is additionally listed as "negative" for skin prick and oral challenges (http://www.allergome.org). Because Bt Cry proteins are generally heat labile, rapidly digestible, not glycosylated and from a non-allergenic source they are generally considered to be non-allergenicity (Ladics et al., 2006).

Table 3. General international databases available for allergen identification and research.*

Name	Address
All Allergy	http://allallergy.net/allergensearch.cfm
Allergen Database	http://allergen.csl.gov.uk
Allergome	http://www.allergome.org
AllFam	http://www.meduniwien.ac.at/allergens/allfam/
Biotechnology Information For Food Safety Database	http://www.iit.edu/~sgendel/fa.htmhttp://www.iit.edu/~sgendel/fa.htm
Food Allergy Research and Resource Program (FARRP)	http://www.allergenonline.org
InformALL	http://foodallergens.ifr.ac.uk
International Immunogenetics Information System (IMGT)	http://imgt.cines.fr
International Union of Immunological Societies (IUIS)	http://www.allergen.org
Structural Database of Allergenic Proteins (SDAP)	http://fermi.utmb.edu/SDAP/index.html

*Does not include motif analysis tools. Refer to Gendel, 2009 for a recent review on the scope and use of allergen databases.

Food allergy: A number of studies assessing the allergic potential of samples derived from Bt maize have been published and to date no allergic reaction specific to the Cry proteins has been reported (Table 4).

Table 4. Allergenicity studies on Bt Cry proteins.

Reference	Cry protein	Conclusion
Nakajima et al., 2006	Cry1Ab	“”IgE antibodies specific to Cry1Ab were not found in the sera of Japenese patients with food [*corn*] allergies”
Xu et al., 2009	Cry1Ab & Cry1Ac	“There is a reasonable certainty of no harm resulting from the inclusion of the Cry1Ab/Ac proteins in human food or animal feed.”
Batista et al., 2005	Cry1Ab (MON810, Bt11, T25 & Bt176)	“None of the individuals undergoing tests reacted differentially to the transgenic and non-transgenic samples under study.”
Son, 2006	Cry1Ac	“GM rice with modified Cry proteins has no differences in its protein composition or allergenicity relative to commercial rice.”
Ladics et al., 2006	Cry1F	“Data indicate lack of allergenic concern for Cry1F.”
Takagi et al., 2006	Cry9C	“In this pilot study significant levels of IgE antibodies specific for the three proteins [*also tested PAT & CP4-EPSPS*] were not detected in the sera of Japanese food-allergy patients.”
Raybourne et al., 2003	Cry9C (Starlink)	“None of the adverse advent sera were found to be reactive with recombinant Cry9C antigens. Results do not support the occurrence of allergic reactions to Cry9C, although such reactions cannot be ruled out.”

Pollen allergy: No scientific literature could be found on the assessment of allergenicity in Bt maize pollen. A widely publicised claim, at an anti-GM meeting in Kuala Lumpur, Malaysia in 2004, that Bt maize pollen (Dekalb 818 YG, a derivative of MON810) was responsible for allergic reactions in the Philippines (Anonymous, 2004) has as far as we could determine not been followed up with a scientific publication.

Conclusions

Although maize allergies are not common its potential to induce allergic reactions is recognised and a number of allergens have been identified in maize food and pollen. In contrast, *Bacillus thuringiensis* and specifically its Cry proteins are generally considered to be non-allergenic.

Theoretically, a possible allergic reaction to a particular Bt maize cultivar would therefore be more likely caused by a known maize allergen than the Cry protein. However, this does not exclude the possibility of a chance mutation to the Cry protein (or any other maize protein for that matter) that could somehow cause it to be more allergenic and should therefore be investigated on a case-by-case basis.

Finally, in the unlikely event that the allergenicity of a particular Bt Cry protein in a particular Bt maize cultivar is confirmed, it should be treated in exactly the same way as, for example, Zea m 1 allergenicity – it would be just another, specific allergen that should be managed according to its epidemiology and has no “general GM” reference or implications.

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