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How much does transgenesis affect wheat allergenicity? Assessment in two GM lines over-expressing endogenous genes

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ABSTRACT

Wheat kernel albumins/globulins (A/G) and gluten proteins are responsible for baker's asthma and food allergy in atopic subjects. Although no commercial genetically modified wheats are currently being grown, they are under study and the allergenicity of GM products is a major concern.

In order to establish the expected and unexpected effects of genetic transformation on allergenicity and also to carry out a safety assessment of genetic transformation, two GM wheat lines (bread and pasta wheat) transformed with endogenous genes were compared to their untransformed counterparts (*wt*), first by an allergenomic approach, and second, using ELISA with sera from patients suffering from food allergy to wheat and baker's asthma. The 2D immunoblots performed on sera from patients suffering from food allergy and baker's asthma on the A/G fraction of the four lines (two GM and two *wt*) revealed comparable IgE-binding profiles. A total of 109 IgE-binding spots were analyzed by mass spectrometry, and most of the proteins identified had already been described as allergens or potential allergens. Only two IgE-binding proteins were specific to one GM line. The concentration of specific IgE against the A/G fractions of GM wheat lines and their *wt* genotypes differed for some sera.

Biological significance: The originality of our paper is to relate the transformation of wheat lines with their potential allergenicity using patient sera, such focus has never been done before in wheat and should be of interest to the researches working in this field.

Another interesting point of this paper is the study of two types of allergies (respiratory and food) on two wheat genotypes and their GM which reveals that some allergens already known in respiratory allergy could be involved in children suffering from wheat food allergy. In this paper we used a classical 2D proteomic analysis and the protein identifications were performed by mass spectrometry after spot picking and in gel trypsin hydrolysis. Concerning the LC–MS/MS analyses classical software and parameters were used as described in Material and methods. We worked on wheat which is actually not fully

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sequenced that was a difficulty; we therefore searched against two databanks (proteins and ESTs) in order to compare the results. Moreover all proteins reported in our paper were identified with at least three unique peptides.
The identified proteins were checked for their potential allergenicity. In order to have a best interpretation of protein identified in terms of potential allergens, BLAST alignments were performed by using an allergen databank (SDAP). This allows the determination of the cross-reactivity of these identified proteins with known allergens of other species and also the prediction of a potential allergenicity.

1. Introduction

The use of genetic engineering for plant improvement and food production is becoming a common practice with a record increase in the area designated for biotech crops between 1996 and 2010, making GM crops the fastest adopted crop technology in the history of modern agriculture [1]. However, concerns about the unintended effects of gene insertion (such as increased toxicity or allergenicity) necessitate a safety assessment of foods derived from GM plants, which is currently regulated by the Codex Alimentarius (ftp://ftp.fao.org/es/esn/food/guide_plants_en.pdf). This document includes a specific paragraph addressing the question of allergenicity, the modified content of known allergens and the appearance of new and unknown allergens for GM plants. Moreover, the European Food Safety Authority (EFSA) recommends comparing GM food with its appropriate counterpart, when the recipient of the introduced gene is known to be allergenic [2]. Bioinformatic tools can be used to predict the potential allergenicity of proteins, based on known IgE binding sites of allergens [3–6], but are ineffective for a safety assessment of genotypes containing potential allergenic proteins that are not well characterized.

Wheat is an important part of the daily diet of millions of people, with a total production of about 600 million tons per year, 75% of which is used for food [7]. About 95% of the wheat cultivated worldwide is bread wheat, *Triticum aestivum*, with an AABBDD genome, whereas 5% is durum wheat, *Triticum durum*, whose genome is AABB [8]. The distinctive feature that makes wheat unique compared to other cereals is the viscoelastic properties of its dough, which determine its capacity to give a specific end-product [9]. However, this staple food is one of the six major food allergens [10,11]. Wheat grain proteins are classically subdivided into the water/salt-soluble fraction (including albumins and globulins, A/G), which represents about 20% of the total amount of proteins, and the water/salt-insoluble gluten, which contains gliadins (Gli) and glutenins (Glu) [12]. Gliadins are monomeric proteins that can be broken down into three categories: α/β gliadins, γ-gliadins and ω-gliadins. Glutenins are subdivided into high- and low-molecular weight glutenin subunits (HMW-GS and LMW-GS, respectively) [13]. Most gluten proteins, as well as some of the A/G fraction, are responsible for triggering food allergic reactions [13–15]. Moreover, the latter are also responsible for one of the most common types of occupational allergy, known as baker's asthma [16,17]. The officially recognized allergens are listed in the WHO/IUIS Allergen Nomenclature (www.allergome.org). Even if some gliadins have also been identified as respiratory allergens [18], they are less relevant than those found in the A/G fraction.

Although no commercial GM wheat is currently grown anywhere in the world, there is an increasing interest in this alternative procedure to classic plant breeding. Whatever the target of the transformation, assessment of the safety of such new GM wheat genotypes is essential as it may induce a change in the expression of endogenous allergens. Such studies have been reported for other GM crops, such as rice, soybean and maize [19–22].

In this study, we evaluated the expected and unexpected effects of two different genetic transformation lines on wheat food and respiratory allergenicity: first a GM bread wheat line (based on the cultivar Bobwhite) obtained through the overexpression of an endogenous wheat gene coding for a LMW-GS [23], a class of proteins known to be allergenic [24,25], and second, a durum wheat line (based on the cultivar Svevo) overexpressing the endogenous *Wx-B1* gene encoding a granule-bound starch synthase [26]. In both cases, the transgenes are only expressed in the endosperm. These lines are referred to in the text as: BW-*wt* for the untransformed Bobwhite genotype, BW-GM for the transgenic line, SV-*wt* for the untransformed Svevo genotype and SV-GM for the transgenic line. The determination of the composition of the three main protein classes (A/G, Gli and Glu) revealed large variations that could potentially induce differences in IgE reactivity. In the case of BW-GM, the reactivity of the Glu class was specifically examined because LMW-GS proteins have already been defined as allergens (Tri a 36, http://www.allergen.org). We used an allergenomic approach to identify IgE-binding polypeptides in the A/G fraction of both the transgenic lines and compared them to their wild-type parents. Finally, for both genotypes Svevo and Bobwhite, we also examined the IgE-binding potential against each A/G fraction as it was particularly affected by the two genetic transformation events.

2. Material and methods

2.1. *Patient sera*

Sera were obtained from 21 patients with clinically documented baker's asthma (BA) or a food allergy (FA). The patients' clinical data (symptoms, age, wheat cv Récital-specific IgE) are summarized in Table S1. Sera were obtained from the Service of Clinical Immunology and Allergology at Epinal Hospital, France, and the University Hospital of Udine, Italy, with the informed