Immune analysis of *cry1Ab*-genetically modified potato by in-silico analysis and animal model

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Abstract The safety of feeding transgenic potato (*Solanum tuberosum*), resistant to the potato tuber moth, to Wistar rats was examined from an immunological perspective. The genetically modified potato (GMP) was harbouring *cry1Ab* and *nptII* genes as target and selectable marker genes, respectively. In-silico analysis reconfirmed that Cry1Ab and NPTII protein sequences have no significant homology to known toxins or known allergens. The Wistar rats were fed a diet containing 20% GMP or its parental control, non-GMP (NGMP), for 90 days. The consumption of GMP food did not affect the growth rate, food intake, food efficiency, and general health status of the rats. There were no significant differences in the serum concentrations of immunoglobulin A (IgA), IgG, IgM, interleukin 6 (IL-6), and IFN- γ between GMP and NGMP-fed rats. Based on such data, it is concluded that the transgenic potato had no adverse effect on immunity functions of Wistar rats.

Keywords Cry1Ab · Genetically modified potato · Food safety · Immunity · NPTII · Rat

Introduction

Plant genetic engineering has opened new insights into the development of genetically modified (GM) crops, such as maize, soybean, potato, and cotton with improved agro-nomically traits [1]. One of the most important applications of transgenic technology is the development of resistant crops to insect pests by introducing insecticidal *cry* genes from the soil bacterium *Bacillus thuringiensis* (Bt) [2]. Bt crops (in alone or stacked traits) are reported to occupy more than 43% of global biotech areas [3].

The major goals of GM crops technology are higher crop production and more nutritious food without the use of pesticides. However, the safety concerns of GM food are determination factors for their acceptance in the market, as defined by scientific and regulatory agencies (e.g., ILSI/IFBC, FAO/WHO, EFSA/Codex and OECD), to prevent the introduction of known allergens (from any source) in a food crop that did not contain that protein [1, 4].

Genetic engineering introduces new genes in the food crops, and the resulting new proteins could act as an allergen or toxin in GM food [5]. Moreover, the inserted transgens could change the cellular metabolism in unintended and unanticipated ways, which could lead to production of allergens or toxins in GM food [6].

Therefore, the evaluation of immunotoxicological effects of whole GM food and the potential allergenicity of the purified recombinant proteins sometimes can help regarding the safety assessment of GM food [7].

A ‘weight of evidence’ method, containing sequence similarity to known allergenic proteins using bioinformatics analysis, in vitro digestibility, and animal models, is recommended by the Codex Alimentarius Guidelines to define the risk of allergenicity of GM food [8].

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The prerequisite step for assessing the potential allergenicity of a novel protein is to use bioinformatics tools [8]. In-silico sequence analysis can be used to assess whether the novel protein is a known allergen or not. More than 35% identity over at least 80 contiguous amino acids can be considered as a known allergen, and there is a potent cross-reaction with an existing allergen [9–11].

Routinely, the allergenicity study of GM food is based on the potential allergenic assessment of pure recombinant proteins. However, a few recent studies have investigated the possibility of immunotoxicological effects of whole GM food given to rats at different times [4, 7]. For this reason, the European Commission (EC) project SAFOT-EST ('New methods for the safety testing of transgenic food') conducted an experiment with rats fed a diet containing transgenic rice expressing an insecticidal protein as an important factor for the immunotoxicological assessment of transgenic food [7].

In the present study, we have evaluated the immunotoxicological effects of transgenic potato plants producing recombinant Cry1Ab and NPTII (neomycin phosphotransferase) proteins. This Bt potato is produced to deal with the potato tuber moth (PTM) pest. The Cry1Ab and NPTII expressed in the Bt potato have been studied for potential allergenic cross-reactivity by sequence alignment searches by using bioinformatic tools. Moreover, we have investigated the immunotoxicological potential of the transgenic potato by producing Bt and NPTII toxins in rats that were fed a diet with transgenic or non-transgenic potatoes for 90 days.

Materials and methods

Bioinformatics analysis

A sequence similarity search was conducted against two allergen-specific databases: Allergen Online of Food Allergy Research and Resource Programme (FARRP; <http://www.allergenonline.com>) and Structural Database of Allergenic Proteins (SDAP; <http://fermi.utmb.edu>).

Full-length FASTA search

Structural homologies shared between query sequences and the ALLERGEN sequence database were assessed using the FASTA algorithm. FASTA comparisons were initiated by using the default search and scoring the criteria of Pearson and default-scoring matrix BLOSUM50 [12, 13]. The BLOSUM50 matrix recognized blocks of conserved residues that are at least 50% similar. The extent of homology was estimated as the percentage similarity and expectation score (E score). The E score indicated the

degree of homology between a pair of sequences based on identity matches or functional similarities and structurally-related similarities. FASTA alignment was conducted to compare the possible contiguous amino acid segments of the proteins against the listed sequences in the databases [8]. Using sliding 80mer search, all possible contiguous 80-amino acid sequences of each query protein was searched, beginning with amino acids 1–80, then 2–81, 3–82 and so on until the last amino acid segment of each protein was compared. We defined the potential allergen when a >35% similarity over segments as short as 80 amino acids with known allergen(s) indicated in the alignment of query, and it should be subjected to further in vivo and in vitro testings [14].

Structural database of allergenic proteins (SDAP)

In addition to the FASTA comparisons used to assess the overall structural similarity, conserved properties of amino acid side chains were applied to identify regions of known allergens similar to peptides from the SDAP database of IgE epitopes. SDAP is a Web server that integrates a database of allergenic proteins (PDB, SWISS-PROT, PIR, GenBank) and the literature (PubMed, MEDLINE) with various bioinformatics tools for performing structural studies related to allergens and characterization of their epitopes. It provides rapid, cross-referenced access to the sequences, structures, and IgE epitopes of allergenic proteins. The main SDAP source of allergens is the IUIS list (<http://www.allergen.org>), and alignments are made with FASTA v 3.45.

Plant materials

No-genetically modified (NGMP) (as control plant) and genetically modified potato (GMP) tubers producing Cry1Ab (as a pesticide) and NPTII (as selectable marker gene) proteins were grown in greenhouse conditions [15]. A light inducible phosphoenolpyruvate carboxylase (PEPC) promoter was induced the Cry1Ab expression, only under light conditions in transgenic potato plants. The harvested tubers were treated by lights ($60 \mu\text{mol}/\text{m}^2/\text{s}^1$) for the Cry1Ab expression in order to be compared with the dark-treated tubers in the feeding tests. The light- and dark-treated (LT and DT, respectively) potato tubers were sliced, dried, and powdered and then used for making experimental diets in accordance with NRC guidelines [16]. In the experimental treatment diets, 20% of potato powder replaced an equivalent amount of cornstarch (Table 1).

Characterization of recombinant DNA and protein

The authenticity of transgenic potato plants was confirmed by the polymerase chain reaction (PCR) analysis. Plant

Table 1 Composition of experimental diets for four groups of rats (%) [16]

Compound	%			
	LT-NGMP	DT-GMP	LT-GMP	DT-NGMP
Wheat flour	15	15	15	15
Barley flour	8	8	8	8
Corn flour	16.5	16.5	16.5	16.5
Wheat bran	2	2	2	2
Soybean meal	13	13	13	13
Alfalfa powder	2	2	2	2
NaCl	0.8	0.8	0.8	0.8
Multi vitamin ^a	0.1	0.1	0.1	0.1
Vit E	0.7	0.7	0.7	0.7
Vit D3	0.2	0.2	0.2	0.2
Vit C	0.2	0.2	0.2	0.2
Milk powder ^a	2.5	2.5	2.5	2.5
Meat meal	17	17	17	17
Soy oil	2	2	2	2
DT-NGMP powder	–	–	–	20
LT-NGMP powder	20	–	–	–
DT-GMP powder	–	20	–	–
LT-GMP powder	–	–	20	–

^a In mg/kg diet: Vit. A: 5000 (IU); Vit. E: 50 (IU); Thiamin: 5; Riboflavin: 6; Pyridoxol: 8; Folic acid: 2; D-biotin: 0.3; Vit. B12: 0.03; Panthothenate: 20; Cholinhydrogentartrat: 2600; Inositol: 400; Nicotinic acid: 40; Phylloquinine: 1; p-aminobenzoic acid: 40; Methionine: 1000; L-cystine: 2000

DNA was extracted by Magan Plant DNA Extraction Kits (Gil NanoGene Biotech, Iran) based on the manufacturer's instruction.

PCR reactions were conducted using 25 ng of template DNA in 25 µl of 50 mM KCl, 10 mM of Tris-HCl (pH 8.3), 1.5 mM of MgCl₂, 200 µM of dNTPs, 50 pM of each primer pairs (F-cry1Ab: 5'atc gagaccggctacacc3', R-cry1Ab: 5'gaggtggcagctgtgttc3' for *cry1Ab* and F-nptII: 5'gctattcggtatgactgggcac3', R-nptII: 5'tcatctgatcgacaa gaccggc3' for *nptII* genes), and 1 U Taq DNA polymerase (Roche, Germany). The reaction mixture was subjected to an initial denaturation cycle of 94 °C for 5 min, 36 cycles each of denaturation [1 min (30 s for *nptII*) at 94 °C], annealing [1 min (30 s for *nptII*) at 58 °C], 90 s (30 s for *nptII*) at 72 °C, and a final elongation cycle of 72 °C for 5 min. Finally, PCR products were evaluated on a 1% agarose gel stained with GelRed.

The concentrations of Cry1Ab and NPTII proteins in the rats' diets were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer instructions (Bt-Cry1Ab/1Ac ELISA; complete kit PSP 06200/0288 and *nptII* ELISA;

complete kit PSP 73000/0288; Agdia; Elkhart, IN). Moreover, the presence of Cry1Ab proteins in the rat diet was detected using a lateral flow test strip (Bt-Cry1Ab/1Ac Immuno-Strip; Agdia, Elkhart, IN) according to manufacturer instructions.

Animal feeding trials

Sixteen adult males and 16 adult female Wistar rats (130–180 g) were divided randomly in 16 cages with two males or two females in each cage. The rats were kept at 23 °C with a 12 h light/dark cycle and 50–60% humidity on a basal diet for one week. Then, they were divided into four groups, each containing two cages with male and two cages with female rats. These groups were fed diets containing light-treated genetically modified potato (LT-GMP), dark-treated genetically modified potato (DT-GMP), light-treated non-genetically modified potatoes (LT-NGMP), dark-treated non-genetically modified potatoes (DT-NGMP), respectively. The rats had free access to food and water for 90 days.

The rats were inspected twice daily and their weights recorded weekly. The food consumed by them was recorded for each cage every day. Water consumption was recorded twice a week throughout the experimental period. The animal card was conducted according to the Institutional Animal Care and Use Committee (IACUC) and it was tried to provide good conditions to bring up the rats.

Immunization with SRBC

Sheep red blood cells (SRBC) from a single source were prepared from Razi Vaccine and Serum Research Institute, Karaj, Iran. The SRBC in phosphate-buffered saline (PBS) was washed three times (1000×g, 20 min, +4 °C) and the buffy coat removed. At the end of washing, SRBC concentrations were measured [7]. The rats were immunized by the intraperitoneal (IP) injection with 0.2 mL of 3.4×10^8 SRBC/ml, six days prior to sacrifice.

Finally, the animals were anesthetized by halothane inhalation and killed by exsanguinations. Blood was collected and sera were stored at –20 °C for the next analysis.

Immunoglobulin analysis

To determine the total IgM, IgG, and IgA content, 96-well Microtiter plates (MaxiSorp, Nunc, Roskilde, Denmark) were coated with appropriate dilutions of serum for each immunoglobulin and incubated for 2 h at room temperature (RT). The plates were washed four times with the PBS (0.01 M, pH 7.4), containing 0.05% Tween 20, prior to each subsequent step. The remaining active sites of the Ig blocked with PBS/Tween buffer for 2 h at RT, and the

plates were incubated with 1 µg/mL of the goat polyclonal secondary antibody to rat IgA (Abcam, Cambridge, UK), 1 µg/mL mouse monoclonal secondary antibody to rat IgG (Abcam, Cambridge, UK), 0.5 µg/mL mouse monoclonal secondary antibody to rat IgM (Abcam, Cambridge, UK), and antibodies in PBS and Glycerol, pH 9.6 for 2 h at RT. The plates were incubated for 1 h with horseradish peroxidase (HRP) and then 100 µL H₂PO₄ were added to the plates for staining. After 10 min, the plates were read at 450 nm on an ELISA reader (Bio-Tek Instrument, Inc.). The concentration of Ig was expressed as mg/mL serum.

Cytokine analysis

The levels of serum cytokines were measured using interleukin-6 (IL-6) antibody (Abcam, Cambridge, UK) and IFN (interferon)—γ, (Abcam, Cambridge, UK), and rabbit polyclonal antibody (Abcam, Cambridge, UK) as secondary antibody for detection according to methods explained for the total IgM, IgA, and IgG.

Statistical analysis

Data is presented as mean ± SE, and statistical analysis was performed using SAS Release 9.1 (SAS Institute Inc., Cary, NC). The significance of the differences has been tested using the parametric analysis of variance (ANOVA). Statistics with p-values less than 0.05 were considered significant. Data on males and females was always analysed separately.

Results and discussion

We employed a new strategy for controlling the potato tuber moth in the field stage using a light regulation of *cry1Ab* in transgenic potato plants. However, the constitutive expression of transgenes using constitutive promoters—e.g., cauliflower mosaic virus (CaMV) 35S—is widely used in plant genetic engineering, but this may impose an extra metabolic burden and other metabolic effects on transgenic plants [17]. For this reason, we used a light inducible promoter (PEPC) for the *Cry1Ab* expression in transgenic potatoes [15]. The light-inducible PEPC promoter has specific advantages in plant genetic engineering for green tissue-consuming pests [15]. The application of the PEPC promoter would not only reduce the metabolic pressure imposed on transgenic potato plants, but also reduce concerns over the food safety of transgenic plants [17, 18]. We indicated the light induce expression of *Cry1Ab* protein in green tissues, leaves, and light-treated tubers of transgenic potato plants (Table 2). This expression system could effectively control the PTM pest in

in vitro and greenhouse assays [15]. In the present work, we studied the potential allergenicity of new transgenic potato plants expressing *Cry1Ab* and *NPTII* protein at in-silico and animal model levels.

Bioinformatics analysis

The full length FASTA alignment and sliding 80 amino acid approaches for the protein sequence of *Cry1Ab* and *NPTII* in allergen databases FARRP were assessed. Both the tests were carried out using the default settings of E value (≥ 0.01) and Z score for full length FASTA and a homology of $\geq 35\%$ over 80 amino acid stretches. The results showed no similarity between the two proteins and known allergens owing to E score ≥ 0.01 (Table 2). Matching the 80 amino acid of the FARRP indicated no segment in any of the two *Cry1Ab* and *NPTII* protein sequences with over 35% similarity to all known allergens.

The sequence search analysis of *Cry1Ab* and *NPTII* proteins were examined in another allergen-specific database, SDAP. The highest scoring identity for *Cry1Ab* under full FASTA alignment was found to be 12.77% with Glym Bd 28 K from soybean, followed by Curl 4.0101 (*Curcularia lunata*) (8.95%) and Solat 1 from (*Solanum tuberosum*) (both 8.98%) (Table 2). Fungi-derived allergens are dominant among allergens with less similarity.

The full FASTA alignment for *NPTII* resulted in a highest of 11.68% similarity with the Min nt1 allergen from *Mimachlamys nobilis*, 10.95% with Alta4 from *Alternaria alternata*, 9.85 with Chi K10 from *Chironomus kiiensis*, and 8.76 and 8.39% with Pis v 2.0101 and Pis v 2.0201 (both from *Pistacia vera*), respectively (Table 2). In addition, the results showed two little similarities (8.76 and 3.28%) between *NPTII* and Asp f11 from *Aspergillus fumigates* and Ant o 1.0101 from *Anthoxanthum odoratum*, respectively (Table 2).

Sequence homology between a novel protein and a known allergen is likely to cause cross-reactions, when there are more than 35% similarity over a segment of 80 or greater amino acids [9]. However, the structures with low-sequence homology may also cause cross-reactivity due to the structural similarity [9]. In the present study, the similarity between *Cry1Ab* and *NPTII* to all known allergens was significantly lower than 35%.

Our findings are in accordance with the earlier study by Mathur et al. [1] on the safety assessment of *Cry1Ab*, *Cry1Ac* and *Cry1C* proteins using in silico tools, where they predicted them as safe for incorporation in GM Maize.

In the present study, the in silico analysis of *Cry1Ab* protein used in the transgenic potato indicated no evidence for any similarity between *Cry1Ab* and any known toxic or allergenic proteins. Thus, our findings confirm the findings of earlier workers [1, 8, 19]. Moreover, the bioinformatic

Table 2 Sequences Homology Analysis for Cry 1AB and NPTII proteins sequences using FASTA against allergen databases

Query sequence	Accession No.	80 amino acid sliding window approach Allergen Online FARRP	Full Length FASTA Search SDAP
Cry1Ab	AX441371	No hits >35% identity found (E score ≥ 0.01)	12.77%(54/423) Gly m Bd28 K (BAB21619) 8.98 (38/423) Cur 1 4.0101(ACF19589) 8.98 (38/423) Solat 1(CAA27571) 8.51 (36/423) Tria gliadin (AAA34285) 8.27 (35/423) Tria gliadin (AAA34286) 8.27 (35/423) Eum 3 (O97370) 7.80 (33/423) Copc 7(CAB52133) 7.09 (30/423) Lig v1(O82015) 6.15 (26/423) Bran 1(P80208) 4.73 (20/423) Pench 20.0101(AAB34785) 4.02 (17/423) Vesv 2.0201(AAX14718)
NPTII			11.68 (32/274) Mimn 1 (AAG08989) 10.95 (30/274) Alta 4 (CAA58999) 9.85 (27/274) Chik 10 (CAA09938) 8.76 (24/274) Pisv 2.0101 (DQ631676) 8.76 (24/274) Aspf 11 (CAB44442) 8.39 (23/274) Pisv 2.0201 (DQ631677) 3.28 (9/274) Anto 1.0101 (Q7M1X6)

analysis of NPTII protein in transgenic potato plants showed no significant alignment and homology at the domain level with proteins known to be toxic or bioactive. Thus, based on the in silico finding, Cry1Ab and NPTII proteins are safe for use in GMO food. The NPTII protein is ubiquitous in the environment (e.g., in bacteria); it is present in many previously authorized genetically modified crops in the world (www.isaaa.org). NPTII safety was studied by many researchers, and their data confirm that the NPTII protein is unlikely to be a toxin or allergen [20–22].

Authenticity of the diets

The composition and nutrient content of all diets was similar regardless of the identity of the potato tubers (Table 1). The only difference between GMP and NGMP diets was the presence or absence of transgenic potato. The validity of the diets was checked by molecular analysis. *nptII* and *cryIAb* transgenes were identified in all diets containing transgenic potatoes by the PCR analysis. This indicating that the transgenes were stably inherited by in vitro and greenhouse growth. Moreover, it confirmed the authenticity of transgenic and non-transgenic potato diets (Fig. 1).

The amount of Cry1Ab protein detected by ELISA in diets formulated with 20% LT-GMP was 49.3 ng/g (fresh wt) (Table 3). The Cry1Ab protein was not detected in the

Fig. 1 PCR analysis of transgenic and non-transgenic diets using specific primers of *cryIAb* (A) and *nptII* (B) genes. 1 1 kb ladder, 2 pPEP-Cry1Ab plasmid (positive control), 3 water (negative control), 4 diet containing LT-GMP, 5 diet containing DT-GMP, 6 diet containing LT-NGMP; diet containing DT-NGMP. (GMP genetically modified potato, NGMP, non-genetically modified potato, LT light-treated, DT dark-treated)

diets containing DT-GMP, DT-NGMP, and LT-NGMP by ELISA. Moreover, the immunoassay analysis using immunochromatographic strips detected the Cry1Ab protein in the diets containing LT-GMP, but it was not detectable in the diets containing DT-GMP, DT-NGMP, and LT-NGMP (Fig. 2).

Table 3 ELISA of Cry1Ab and NPTII proteins in the diets containing transgenic potato

Diet	Cry1Ab (ng/g)	NPTII (ng/g)
LT-NGMP	0 ^b	0 ^b
DT-NGMP	0 ^b	0 ^b
LT-GMP	49.8 ± 3.9 ^a	55.15 ± 2.4 ^a
DT-GMP	0 ^b	53.97 ± 2.1 ^a

Data is presented as group mean values ± SE

(GMP genetically modified potato, NGMP, non-genetically modified potato LT light-treated, DT dark-treated)

The values in columns with different letters are significant at $p \leq 0.05$

Concentrations of the NPTII protein in the diets containing LT-GMP and DT-GMP were 55.15 and 53.97 ng/g (fresh wt), respectively (Table 3). The NPTII protein was not detected in the diets containing NGMPs.

Rat performance

The rats in all groups were determined to be in good health throughout the experiment. There were no significant differences in general health and the growth rate of the rats between the groups (Table 4). However, we observed the naturally significant differences between male and female rats' weights and food and water consumption. Light-treated potatoes significantly affected the food and water consumption, but their body weight was not affected by light elements. Moreover, there was no significant differences in the body weight and, food and water consumption between rats that were fed GM potatoes and non-GM potatoes (Table 4).

Fig. 2 Immunostrip assay for Cry1Ab protein in the GIT contents of rats fed on rats diets. 1- LT-NGMP, 2- DT-NGMP, 3-DT-GMO, 4-LT-GMP (LT light treated, DT dark treated, NGMP, Non-genetically modified potato, GMP genetically modified potato). The control band confirms the correct function of the immune-strips; the Cry1Ab band shows the presence of Cry1Ab protein

Table 4 Effects of feeding diets containing GMP on body weight, food intake, and food efficiency of experimental groups at the end of the experimental periods (90 days)

SKM	Male				Female			
	NGMP		GMP		NGMP		GMP	
	L	D	L	D	L	D	L	D
Initial body weight (g)	166.6 ± 10.7 ^a	162.9 ± 15.7 ^a	164.3 ± 12.8 ^a	165.1 ± 23.4 ^a	133.6 ± 13.5 ^b	136 ± 11.2 ^b	134 ± 12.8 ^b	133.9 ± 13.5 ^b
Final body weight (g)	334.9 ± 25.3 ^a	305.6 ± 30.4 ^a	338.1 ± 28.4 ^a	318.9 ± 17.1 ^a	200.3 ± 11.9 ^b	211.4 ± 12.2 ^b	197.3 ± 18.9 ^b	206.5 ± 9.44 ^b
Daily body weight gain (g)	1.87 ± 0.12 ^a	1.58 ± 0.10 ^a	1.93 ± 0.09 ^a	1.71 ± 0.73 ^a	0.74 ± 0.06 ^b	0.84 ± 0.07 ^b	0.71 ± 0.05 ^b	0.81 ± 0.06 ^b
Food Intake	19.3 ± 1.59 ^a	17.8 ± 0.68 ^a	17.8 ± 0.60 ^a	19.8 ± 0.87 ^a	13.5 ± 1.23 ^b	14.1 ± 0.83 ^b	11.8 ± 0.78 ^b	13.3 ± 1.2 ^b
Food efficiency (%)	1.03 ± 0.01 ^a	1.13 ± 0.01 ^a	0.92 ± 0.01 ^a	1.16 ± 0.02 ^a	1.82 ± 0.01 ^b	1.68 ± 0.011 ^b	1.66 ± 0.01 ^b	1.64 ± 0.01 ^b

Data are presented as group mean values ± SE for 8 rats

GMP genetically modified potato, NGMP non genetically modified potato, L light treated potato, D dark treated potato

The values in rows with different letters are significant at $p \leq 0.05$

Table 5 Effect of GMP diets on the concentration (mg/mL) of serum immunoglobulins (IgA, IgM, IgG) and cytokines (IL-6 and IFN- γ) rats after 90 days of feeding

Rat	Diet	IgA	IgM	IgG	IL-6	IFN- γ
F	LT-NGMP	0.58 $\pm$ 0.06 ^a	1.23 $\pm$ 0.28 ^a	2.07 $\pm$ 0.36 ^a	0.74 $\pm$ 0.06 ^a	0.61 $\pm$ 0.02 ^a
	DT-NGMP	0.68 $\pm$ 0.28 ^a	1.2 $\pm$ 0.36 ^a	1.97 $\pm$ 0.20 ^a	0.77 $\pm$ 0.16 ^a	0.65 $\pm$ 0.14 ^a
	LT-GMP	0.66 $\pm$ 0.28 ^a	1.31 $\pm$ 0.40 ^a	2.13 $\pm$ 0.40 ^a	0.7 $\pm$ 0.05 ^a	0.61 $\pm$ 0.06 ^a
	DT-GMP	0.73 $\pm$ 0.30 ^a	1.37 $\pm$ 0.28 ^a	2.17 $\pm$ 0.26 ^a	0.69 $\pm$ 0.08 ^a	0.59 $\pm$ 0.05 ^a
M	LT-NGMP	0.82 $\pm$ 0.18 ^a	1.15 $\pm$ 0.25 ^a	1.85 $\pm$ 0.46 ^a	0.52 $\pm$ 0.08 ^a	0.45 $\pm$ 0.08 ^a
	DT-NGMP	0.75 $\pm$ 0.25 ^a	1.17 $\pm$ 0.34 ^a	2.02 $\pm$ 0.32 ^a	0.63 $\pm$ 0.11 ^a	0.55 $\pm$ 0.09 ^a
	LT-GMP	0.68 $\pm$ 0.11 ^a	0.88 $\pm$ 0.44 ^a	2.08 $\pm$ 0.28 ^a	0.68 $\pm$ 0.14 ^a	0.52 $\pm$ 0.08 ^a
	DT-GMP	0.69 $\pm$ 0.24 ^a	0.71 $\pm$ 0.31 ^a	2.04 $\pm$ 0.27 ^a	0.62 $\pm$ 0.15 ^a	0.52 $\pm$ 0.10 ^a

Data is presented as group mean values $\pm$ SE

The values in columns with different letters are significant at $p \leq 0.05$

F female, M male, LT light treated, DT dark treated, NGMP non-genetically modified potato, GMP genetically modified potato

A similar analysis was conducted by El-Sanhoty et al. [6] on rats that were fed transgenic potato plants containing *cryV* genes. They found no statistical difference in food intake, daily body weight gain, and feeding efficiency between the control group and the rats that were fed transgenic potato tubers [6]. The same results were obtained by Zdunczyk et al. [23] on the rat diets containing transgenic potato plants resistant to potato virus Y (PVY). There were no differences between transgenic and non-transgenic plants in estimated indices of nutritive value [23].

Immunoglobulins and cytokines levels

In general, there were no statistically significant differences in the concentration of total IgA, IgM and IgG, IL-6, and IFN- γ in serum between the groups that were transgenic and non-transgenic potatoes (Table 5).

The immune assessment of GM food is based on the potential allergenic evaluation of the newly produced protein, and the whole food is not considered [7]. In the present work, we studied the immunological assessments of the whole food due to the potential unintended effects arising from the general mutagenic nature of the genetically modification process.

Specific antibodies or immunoglobulins are produced by B lymphocyte cells during an antigen-stimulated immune response [24]. IgG is the main immunoglobulin in serum. In persons susceptible to non-atopic disease, the immune response is linked with the production of specific IgG for extrinsic proteins and neutralizing protein antigens in serum, while its enhanced concentration reveals the defensive response of a body [25].

The early stages of the antibody response are dominated by IgM produced by B cells before activation. Later, IgG and IgA are the predominant isotypes, contributing a small but biologically important part of the response [26, 27].

The production of IgG and IgA is mainly dependent on an antibody switch from IgM, an active process driven to a large extent by the cytokine environment [28].

In addition, IFN- γ modulates IL-6 signalling through its soluble receptor [29]. In mammals, IL-6 is a multifunctional cytokine that induces the degranulation of neutrophils, activates macrophages and T lymphocytes, and stimulates the differentiation of normal B lymphocytes to antibody-producing cells [30, 31]. This pro-inflammatory cytokine, IL-6, is a modulator of the levels of IgG, IgM, IgA and some complements [32, 33].

In the present study, no significant differences were found with respect to the serum levels of IgA, IgM, IgG, IL-6, and IFN- γ between rats that were fed GMP and NGMP. Moreover, there was no significant differences among IgA, IgM, IgG, IL-6 and IF- γ levels for male and female animals.

Feeding transgenic *cryIAb/cryIAc* rice indicated no effect on the concentrations of IgY and IgM in broilers [34]. Song et al. [24] reported no effect on IgG, IgM, and IgE levels in mice-fed GM corn, while Kroghsbo et al. [7] found that most immune parameters had no significant change, except IgG, in rats that were fed *Bt* rice. However, a dose-dependent effect of GM rice on the level of IgA was reported by them.

The increasing levels of serum IL-6, IL-13, IL-12p70, and MIP-1 is found as a peripheral immune response in mice-fed GM maize MON810 (harboring *cryIAb* gene) [4]. However, Liu et al. [34] reported no significant effects on serum IL-4 and IL-6 in broilers-fed transgenic rice.

In conclusion, according to EFSA [35], the consumption of CryIAb containing food is regarded safe. Based on in silico tools, we reconfirmed that CryIAb and NPTII protein sequences are non-allergenic sequences. Moreover, compared with NGMP diet, GMP diet had no significant effect on body weight and serum levels of IgA, IgM, IgG, IL-6, and IFN- γ for 90 days in rats. No adverse immunological

effects were found for either the transgenic potato or the corresponding recombinant protein after 90 days of the feeding trial. Therefore, the GM potato may be as safe as its native counterpart with reference to allergenic responses. This data can be used as additional information for risk analysis of transgenic potatoes and will be used to provide the regulatory authorities as risk assessment documents.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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