



NOVEL FOOD INFORMATION - FOOD BIOTECHNOLOGY

COLORADO POTATO BEETLE AND POTATO LEAFROLL VIRUS RESISTANT POTATO LINES RBMT21-129, RBMT21-350, RBMT22-082

Health Canada has notified Monsanto Canada Inc. that it has no objection to the food use of the transgenic NewLeaf-Plus™ potato cultivars Russet Burbank (RBMT21-129, RBMT21-350 and RBMT22-082), which have been developed to be resistant to the Colorado potato beetle (CPB) and to resist infection by the plant luteovirus, potato leafroll virus (PLRV). The Department conducted a comprehensive assessment of the Russet Burbank NewLeaf-Plus™ potato cultivars according to its *Guidelines for the Safety Assessment of Novel Foods* (September 1994). These guidelines are based upon internationally accepted principles for establishing the safety of foods derived from genetically modified organisms.

BACKGROUND:

The following provides a summary regarding the Monsanto Canada Inc. notification to Health Canada and contains no confidential business information.

1. Introduction

The NewLeaf-Plus™ potato (*Solanum tuberosum*) lines RBMT21-129, RBMT21-350 and RBMT-22-082 were developed through a specific genetic modification of cultivar Russet Burbank to be CPB (*Leptinotarsa decemlineata* Say.) resistant and to resist infection by PLRV. The novel lines produce a version of the insecticidal protein, CryIII_A, derived from *Bacillus thuringiensis*, and the DNA sequences corresponding to open reading frames 1 (ORF-1) and 2 (ORF-2) from PLRV. Delta-endotoxins, such as the CryIII_A protein expressed in Russet Burbank NewLeaf-Plus™ potatoes, act by selectively binding to specific receptors localized on the brush border midgut epithelium of susceptible insect species. Following binding, cation-specific pores are formed that disrupt midgut ion flow and thereby cause paralysis and death. CryIII_A and related endotoxins are insecticidal only to lepidopteran or coleopteran insects and their specificity of action is directly attributable to the presence of specific receptors in the target insects. There are no receptors for delta-endotoxins of *B. thuringiensis* on the surface of mammalian intestinal cells, therefore, livestock animals and humans are not susceptible to these proteins. PLRV is a spherical RNA virus belonging to the luteovirus group and is transmitted primarily by the green peach aphid (*Myzus persicae*). This virus has a worldwide distribution and commonly infects potato causing serious disease and economic loss. The introduced viral sequences do not result in the formation

This Novel Food Information document has been prepared to summarize the opinion regarding the subject product provided by the Food Directorate, Health Protection Branch, Health Canada. This opinion is based upon the comprehensive review of information submitted by the petitioner according to the *Guidelines for the Safety Assessment of Novel Foods*.

(Également disponible en français)

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of any infectious particles, nor does their expression result in any disease pathology. The genetically modified potato cultivars exhibit the trait of resistance to infection and subsequent disease caused by PLRV through an incompletely understood process which may involve silencing of viral gene translation, which has been termed “replicase-mediated resistance”.

2. Development of the Modified Plant

The transgenic Russet Burbank potato lines RBMT21-129, RBMT21-350 and RBMT22-082 were created through two separate *Agrobacterium*-mediated transformation events in which the transfer DNA (T-DNA) contained the genes encoding the CryIII_A protein from *B. thuringiensis* subsp. *tenebrionis* and the ORF-1 and ORF-2 regions from PLRV. These two ORFs encode the putative viral helicase and replicase domains that are required for viral RNA synthesis. In addition, the T-DNA contained sequences encoding the enzymes neomycin phosphotransferase II (NPTII) or 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The respective expression of either NPTII or EPSPS activity in RBMT21 and RBMT22 transformation events was used as a selectable trait for screening transformed plants for the presence of the *cryIII_A* and PLRV replicase genes. There was no incorporation of plant translatable plasmid DNA sequences outside of the T-DNA region and stable inheritance of the novel traits was demonstrated over multiple generations of vegetative propagation.

3. Product Information

The constitutive expression of CryIII_A protein was demonstrated in each of the transgenic NewLeaf-Plus™ Russet Burbank cultivars at levels comparable with previously approved NewLeaf™ potato cultivars. The production of viral replicase protein in RBMT21-129, RBMT21-350 and RBMT22-082 was undetectable in either leaf or tuber tissue, and is, therefore, likely to be less than for plants naturally infected with PLRV. The level of expression of NPTII or CP4 EPSPS in all plant tissues from RBMT21-129 or 350 and RBMT22-082, respectively, was comparable to the amounts measured in previously approved novel plants expressing either of these proteins. The presence of NPTII protein has been judged to be insignificant with respect to any human health risk due to exposure. Solanine and chaconine are the principal glycoalkaloids commonly found in potato tubers. The concentration of total glycoalkaloids (TGA) in NewLeaf-Plus™ Russet Burbank tubers ranged between 4.3-17.1 mg/100g fresh weight tissue, with a mean value of 6.7 mg/100g fresh weight tissue. This is comparable to the TGA range of 2.5-16.1 mg/100g fresh weight tissue that was previously measured in tubers from commercial non-transgenic cultivars of Atlantic, Gemchip, Norchip and Russet Burbank. In each case, the TGA concentration in transgenic tubers was below the administrative guideline of 20 mg/100g fresh weight that has previously been established for TGA in potato. Other than resistance to CPB and infection by PLRV, the disease, pest and other agronomic characteristics of the NewLeaf-Plus™ Russet Burbank lines were comparable to the non-transgenic Russet Burbank cultivar.



4. Dietary Exposure

Potatoes are considered to be a staple food for many Canadians, constituting up to 37% of the total average vegetable intake. The genetic modification present in the RBMT21-129, RBMT21-350 and RBMT22-082 transgenic lines will not result in any change in the consumption pattern for potatoes. Due to their protection from CPB damage and resistance to infection by PLRV, the NewLeaf-Plus™ Russet Burbank cultivars are expected to replace some existing commercial potato cultivars in all potato product applications. Hence, they will provide an alternate or additional choice to consumers and food manufacturers.

5. Nutrition

The analysis of macro- and micronutrients from RBMT21-129, RBMT21-350 and RBMT22-082 transgenic lines revealed only small differences with the respective values from non-transgenic controls and in each case the level was within the normal range of variation reported for commercial potatoes. The consumption of products from NewLeaf-Plus™ Russet Burbank potatoes will have no significant impact on the nutritional quality of the Canadian food supply.

6. Safety

a) Potential Toxicity:

The amino acid sequence of the CryIII_A protein expressed in NewLeaf-Plus™ potatoes is closely related to the sequence of the same proteins that are present in strains of *B. thuringiensis* that have been used for over 30 years as commercial organic microbial insecticides. An analysis of the amino acid sequences of the inserted CryIII_A protein and PLRV replicase did not show homologies with known mammalian protein toxins and they are not judged to have any potential for human toxicity. The history of known safe consumption of PLRV replicase from virus-infected plant products provides additional evidence of lack of toxicity.

b) Potential Allergenicity:

The CryIII_A protein and PLRV replicase do not possess characteristics typical of known protein allergens. There were no regions of homology when the sequences of these introduced proteins were compared to the amino acid sequences of known protein allergens. Unlike known protein allergens, the CryIII_A protein is rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric or intestinal fluids. The CryIII_A protein and PLRV replicase are extremely unlikely to be allergenic.



CONCLUSION:

Health Canada's review of the information presented in support of the food use of CPB and PLRV resistant RBMT21-129, RBMT21-350 and RBMT22-082 potato lines concluded that they do not raise concerns related to human food safety. Health Canada is of the opinion that products from NewLeaf-Plus™ Russet Burbank potato cultivars are as safe and nutritious as those available from current commercial potato cultivars.

Health Canada's opinion pertains only to the food use of these CPB and PLRV resistant potato lines. Issues related to growing NewLeaf-Plus™ Russet Burbank potatoes in Canada and their use as animal feed are addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.