Health Canada Santé Canada

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# NOVEL FOOD INFORMATION - FOOD BIOTECHNOLOGY

## VIRUS RESISTANT SQUASH LINE ZW-20

Health Canada has notified Seminis Vegetable Seeds Inc. that it has no objection to the food use of the transgenic squash line ZW-20, which has been developed to resist infection by two plant viruses that commonly infect squashes, watermelon mosaic virus 2 (WMV2) and zucchini yellows mosaic virus (ZYMV). The Department conducted a comprehensive assessment of ZW-20 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 Seminis Vegetable Seeds Inc. notification to Health Canada and contains no confidential business information.

### 1. Introduction

The yellow crookneck squash (*Curcurbita pepo* L.), line ZW-20, was developed through a specific genetic modification to be resistant to infection by two plant viruses WMV2 and ZYMV, which are members of the potyvirus group. The novel variety was developed by insertion of the coat protein (CP) encoding sequences from these two single-stranded RNA viruses. The introduced viral sequences do not result in the formation of any infectious particles, nor does their expression result in any disease pathology. The genetically modified squash exhibits the trait of resistance to infection and subsequent disease caused by these viruses through a process that is related to viral cross-protection.

### 2. Development of the Modified Plant

The ZW-20 squash line was created by *Agrobacterium*-mediated transformation in which the transfer-DNA (T-DNA) contained the coat protein genes from each of the two viruses. The constitutive expression of these genes was regulated by the 35S promoter from cauliflower mosaic virus (CaMV). In addition, the sequence of the WMV2 coat protein was introduced as a 5'-chimera that included the sequence encoding the N-terminal 16 amino acids of the CaMV coat protein. While the gene encoding neomycin phosphotransferase (NPTII) was incorporated into the Ti plasmid vector used for transformation, only plants lacking this gene were selected for commercialization. The gene encoding NPTII is, therefore, not

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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present in the ZW-20 genome. There was no incorporation of plasmid DNA sequences outside of the T-DNA region as verified by Southern blot analysis.

## 3. Product Information

Constitutive expression of the ZYMV and WMV2 coat proteins in transgenic squash were determined by enzyme linked immunosorbent assay (ELISA) and found to range between 73 – 1218 µg/kg fresh weight and 47-112 µg/kg fresh weight, respectively. This level of expression was significantly lower than measured levels of ZYMV and WMV2 coat protein in zucchini squash, yellow crookneck squash, cantaloupe and honeydew melons from supermarket shelves. These latter levels have been estimated to be up to 268 and 421 fold greater than the corresponding amounts of ZYMV and WMV2 CP produced in transgenic squash. Human consumption of viral coat proteins occurs whenever virus-infected plant products are consumed, or where crops have been protected from virus infection by classical cross-protection using a mild or symptomless strain of the virus. Other than susceptibility to virus infection, the disease, pest and other agronomic characteristics of line ZW-20 were comparable to non-transgenic counterparts.

## 4. Dietary Exposure

The fruit of ZW-20 is intended primarily for human consumption and the genetic modification will not result in any change in the consumption pattern for this product. Consequently, the dietary exposure of Canadians to this product is anticipated to be the same as for other commercially available squash lines.

## 5. Nutrition

The analysis of nutrients from transgenic ZW-20 squash and non-transgenic squash revealed only small differences that were within the range of variability normally reported for squash. The consumption of this product will have no significant impact on the nutritional quality of the Canadian food supply.

## 6. Safety

a) Potential Toxicity:

The WMV2 and ZYMV coat protein sequences were compared to databases of known protein toxins and did not show homologies with known mammalian protein toxins. The history of known safe consumption of these proteins from virus-infected plant products provides additional evidence of lack of toxicity.

b) Potential Allergenicity:

The WMV2 and ZYMV coat proteins 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. The WMV2 and ZYMV coat proteins are extremely unlikely to be allergens.

**CONCLUSION:**

Health Canada's review of the information presented in support of the food use of virus resistant squash ZW-20 concluded that this squash does not raise concerns related to human food safety. Health Canada is of the opinion that ZW-20 is as safe and nutritious as current commercial squash varieties.

Health Canada's opinion deals only with the food use of this virus resistant squash. Issues related to growing virus resistant squash in Canada and its use as animal feed are addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.