# Fusarium Wilt Tropical Race 4 Resistant Banana Event QCAV-4

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## Application for Amendment to Standard 1.5.2 - Food Produced Using Gene Technology

### Executive summary

Banana event QCAV-4 (QUT-QCAV4-6) was developed through recombinant DNA techniques to express a banana disease resistance (R) gene that confers resistance to the fungal disease, Fusarium wilt tropical race 4 (TR4) also known as Panama Disease TR4. TR4 is a devastating disease of bananas which kills the commercially important Cavendish banana in addition to many other banana cultivars including Lady finger. The disease was first identified in Australia in the Northern Territory in 1997 where it has subsequently decimated commercial banana production. In 2015, the disease was detected in the major banana-growing region of North Queensland. Despite the implementation of strict biosecurity protocols, the disease continues to spread. QCAV-4 is not intended to replace the current Cavendish banana cultivars growing in Australia but rather to provide a safety net to the Australian banana industry should it be heavily impacted by TR4. Its approval for release in Australia is likely to open opportunities for the GM banana to be grown in overseas countries where TR4 is having or has the potential to have a devastating impact on banana production.

QCAV-4 was created by *Agrobacterium tumefaciens*-mediated transformation of banana (*Musa acuminata* subgroup Cavendish cv Grand Nain) embryogenic cells with plasmid pSAN3 resulting in the introduction of the *MamRGA2* disease resistance (R) gene from the wild banana *Musa acuminata* ssp. *malaccensis* and the *neomycin phosphotransferase* II (*npt*II) gene from *Escherichia coli* as a plant selectable marker.

The resistance of QCAV-4 to TR4 was evaluated in two OGTR-approved field trials from 2012-2015 (DIR107) and from 2018-present (DIR146). Both trials were conducted in the Northern Territory on a commercial banana farm with high TR4 disease pressure. In both trials, the disease incidence in QCAV-4 plants was significantly lower than the non-genetically modified (non-GM) Grand Nain control plants. Further, except for TR4 resistance, both QCAV-4 plants and fruit were agronomically and phenotypically indistinguishable from the non-GM Grand Nain control plants and fruit.

Molecular characterisation of the introduced genetic material in event QCAV-4 showed the presence of a large single insert mapped to a region on chromosome 6 of the banana genome. Nucleotide sequencing revealed that the insert is comprised of (i) three complete copies of the intended T-DNA, and (ii) two truncated portions of the *MamRGA2* expression cassette. Bioinformatic analysis revealed that no open reading frames in chromosome 6 were disrupted by the insertion. While seven unintended open reading frames (ORFs) resulted from the insertion, none contained the required regulatory elements necessary for expression of mRNA and protein biosynthesis and this was confirmed by RNA-Seq. Analysis of the predicted amino acid sequences from these new ORFs showed that none had the potential to encode a protein with any significant amino acid sequence similarity to known toxins or allergens. Using Southern blot analysis, the introduced genetic material was shown to be stably inherited over five generations of plants. Further, assessment of transgene expression levels showed

that MamRGA2 is providing resistance to TR4 in event QCAV-4 and that the resistance phenotype trait was stable and inherited across multiple generations.

Western blot analysis using a monoclonal mouse anti-MamRGA2 antibody was used to measure the levels of MamRGA2 in fruit and peel tissue collected from QCAV-4 plants, representing the two tissue types with potential pathways of dietary exposure. MamRGA2 protein could not be detected in either fruit or peel tissue (limit of detection: 1-2 ng). Based on the published 2020/21 average Australian annual banana consumption of 16 kg, the maximal exposure to MamRGA2 was therefore calculated to be lower than 8.3 µg/day (assuming 100% of the Australian Cavendish market was replaced with event QCAV-4). Data on the consumption of banana peel in Australia is unavailable but is considered to be marginal in comparison resulting in even lower exposure to the MamRGA2 protein from QCAV-4 peel consumption.

A "weight-of-evidence" approach was followed to assess potential hazards associated with the MamRGA2 protein expressed in QCAV-4. This assessment considered the (i) intra-species source of the *MamRGA2* transgene, (ii) the ubiquitous presence of highly similar R proteins in other food crops and their history of safe consumption in common food crops including banana, (iii) the lack of significant amino acid sequence similarity with known toxins and allergens, and (4) the rapid digestibility of MamRGA2 in simulated gastric fluid containing pepsin. Based on these considerations, it was concluded that further hazard characterisation by animal toxicity testing was not warranted.

The NPTII amino acid sequence expressed in QCAV-4 is nearly identical (99.6%) to that expressed in several GM events already assessed as safe by regulatory bodies in Australia and overseas. Therefore, its safety assessment was limited to (i) an updated bioinformatics comparison of its amino acid sequence to known protein toxins and allergens and (ii) the detection and quantification of the amount of NPTII protein present in edible parts of QCAV-4. Bioinformatic searches found no similarity of NPTII to known or putative protein toxins and allergens. Western immunoblot analysis using a commercially available NPTII-specific antibody revealed the presence of NPTII in both fruit and peel samples collected from event QCAV-4. Using quantitative enzyme-linked immunosorbent assay (ELISA), the average concentration of NPTII in fresh ripe fruit and peel from QCAV-4 was 3.1 and 4.5 ppm, respectively. Based on the published 2020/21 average Australian annual banana consumption of 16 kg, the human dietary exposure to NPTII was calculated at 49.6 mg per year (or 136 µg/day) (assuming 100% of the Australian Cavendish market was replaced with event QCAV-4). Dietary exposure to NPTII through the consumption of QCAV-4 banana peel was difficult to establish because of the lack of reliable data on the consumption of this tissue in Australia. If consumption of banana peel was similar to fruit (16 kg/per/year), the exposure would be 72 mg per year (or 197 µg/day).

Changes in the composition of food derived from QCAV-4 were considered as part of the "weight-of-evidence" approach to examine if there were unintended consequences of the genetic modification in QCAV-4. The levels of proximates (moisture, fat, protein, ash, carbohydrates, and energy), minerals (magnesium, manganese, potassium), and vitamins (ascorbic acid and vitamin B6) in banana fruit and peel tissue were compared between samples collected from both event QCAV-4 and its non-GM counterpart. While there were some statistical differences in the levels of some of the analytes between QCAV-4 and non-GM control, the mean values for proximates, vitamins, and minerals from fruit and peel were mostly within the compositional variation reported in the literature. Further, no consistent pattern indicated that expression of the *MamRGA2* and *npt*II transgenes impacted the nutritional composition of QCAV-4. We conclude from this analysis that event QCAV-4 is substantially equivalent to conventional Grand Nain banana for the levels of all proximates, vitamins, and minerals reported.

Except for resistance to TR4, the analysis of event QCAV-4 presented in this submission has not revealed any biologically relevant differences to the non-GM counterpart, nor could it identify any health and safety concerns, and supports the conclusion that fruit and peel tissue derived from event QCAV-4 is substantially equivalent and as safe as conventional Grand Nain banana. Collectively, results of the molecular characterisation, agronomic assessment and composition analysis support this application for amendment to the *Australia New Zealand Food Standards Code* to allow inclusion of QCAV-4 in **Standard 1.5.2**-*Food Produced Using Gene Technology*.