Food derived using new breeding techniques

William Laing, PhD
Plant and Food Fellow
Plant & Food Research
Food Industry Science Centre
Batchelar Road
Palmerston North 4474, New Zealand
+64-6-925 7686
William.laing@plantandFood.co.nz

Background

I am a retired Plant Scientist who still undertakes research into plant vitamin C at Plant and Food's Palmerston North laboratories. I have published over 100 papers in peer reviewed literature and have a Google H factor of 43. I have most recently undertaken research generating transgenic plants with enriched vitamin C.

In a recent paper we established that a piece of upstream coding sequence [1] (upstream open reading frame or uORF) in front of the gene that controls vitamin C concentrations in plants (called GDP galactose phosphorylase or *GGP*) [2] controlled the production the GGP enzyme and thus controlled the concentration of vitamin C in fruits and other organs. We showed that disabling this uORF by the changing one base pair at its start was sufficient to cause vitamin C to be increased in plants [1]. Thus this is an excellent target for gene editing by CRISPR/Cas and production of fruit and vegetables with high vitamin C. The gene change is dominant in the heterozygous state. We have also shown that over expression of *GGP* also increases vitamin C content in plants [3].

Vitamin C not only has health benefits (up to 23% of US people are vitamin C deficient or depleted), but also stops tissue browning (e.g. fresh cut apples, and an alternative to the RNAi artic apple) and provides stress resistance to many crops. Plant and Food research holds the patent on the use of the gene *GGP*, and on the use of the uORF to control ascorbate.

My answers to questions will be framed in terms of this particular example.

Questions

Do you agree, as a general principle, that food derived from organisms containing new pieces of DNA should be captured for pre-market safety assessment and approval? Yes, the final product whatever the means of production (from simple selection to GMO) should be assessed for safety. This should be based on the product, not on its means of generation. However, the regulatory process should be straight forward and not onerous. In the era of full genome sequencing and sophisticated chemical analysis these should be first steps to doing this. Comparison to existing products should be the basis for decision making.

Should there be any exceptions to this general principle? Product/food safety should be the determining issue. I do not see any exceptions.

Should food from null segregant organisms be excluded from pre-assessment and approval? Not excluded if it is a new product. Some sort of assessment should be made.

If yes, should that exclusion be conditional on specific criteria and what should those criteria be?

If no, what are your specific safety concerns for food derived from null segregants? A general assessment similar to that used for other new breeding products should be made. I have no more concern of recombination of genes producing an unsafe product than I do for conventional breeding.

Are foods from genome edited organisms likely to be the same in terms of risk to foods derived using chemical or radiation mutagenesis? Similar risks. As described above, the gene editing approach could generate high vitamin C plants. Although vitamin C is of very low toxicity, and we already eat fruits with very high vitamin C (grams per day), we should at least know what sort of vitamin C concentration is produced and make a decision whether this new product should be assessed as a medical product. It should be noted that it has been reported that classical mutagenesis has randomly knocked out the uORF mentioned above and generated high vitamin C tomatoes overseas.

If no, how are they different? Not generally different in terms of product. However, gene editing is more precise and defined and gene sequencing can identify any off target results.

If yes, would this apply to all derived food products or are there likely to be some foods that carry a greater risk and therefore warrant pre-market safety assessment and approval? As mentioned above, some foods may carry too high a vitamin C content for some people, and they may need to be warned. However this is no more a risk than e.g. high carbohydrate is to diabetics, and people sensitive to high vitamin C will already be aware of this.

Are you aware of other techniques not currently addressed by this paper which have the potential to be used in the future for the development of food products? Just like Zinc fingers were replaced by Talons, and Talons by CRISPR/Cas, there are sure to be new techniques that will replace CRISPR/Cas. This issue will be no longer the question of detecting the process, but distinguishing the product.

Should food derived from other techniques, such as DNA methylation, be subject to pre-market safety assessment and approval? Only in terms of the product, not the process. As gene expression controls vitamin C content in plants [4] methylation status would probably control levels of GGP and therefore ascorbate. It should be noted that demethylation, at least in animals, is controlled by vitamin C [5] and thus diet may be affecting human and animal methylation status already through this mechanism.

Do you think a process-based definition is appropriate as a trigger for pre-market approval in the case of NBTs? No

If no, what other approaches could be used? Product based. The characterisation of the product by gene sequencing and chemical analysis should be the first steps with comparison to existing similar products. If necessary animal and human trials could be undertaken if concerns are raised by analysis.

If yes, how could a process-based approach be applied to NBTs? Very difficult to implement. Would require new technologies not yet developed.

Are there any aspects of the current definitions that should be retained or remain applicable? As long as the definitions are process based, there will be serious problems

Are there other issues not mentioned in this paper, that FSANZ should also consider, either as part of this Review or any subsequent Proposal to amend the Code? Reduce onerous regulations controlling GM experiments and field trials. I would have no issue with labelling food with the process used to generate it (e.g. organic, conventional, GMO etc)

References

1 Laing, W. A., Martínez-Sánchez, M., Wright, M. A., Bulley, S. M., Brewster, D., Dare, A. P., Rassam, M., Wang, D., Storey, R., Macknight, R. C. and Hellens, R. P. (2015) An Upstream Open Reading Frame Is Essential for Feedback Regulation of Ascorbate Biosynthesis in *Arabidopsis*. The Plant Cell **27**, 772-786

2 Laing, W. A., Wright, M. A., Cooney, J. and Bulley, S. M. (2007) The missing step of the L-galactose pathway of ascorbate biosynthesis in plants, an L-galactose guanyltransferase, increases leaf ascorbate content. Proc. Natl. Acad. Sci. USA **104**, 9534-9539

3 Bulley, S., Wright, M., Rommens, C., Yan, H., Rassam, M., Lin-Wang, K., Andre, C., Brewster, D., Karunairetnam, S., Allan, A. C. and Laing, W. A. (2012) Enhancing ascorbate in fruits and tubers through over-expression of the L-galactose pathway gene GDP-L-galactose phosphorylase. Plant Biotechnol. J. **10**, 390-397

4 Bulley, S. M., Rassam, M., Hoser, D., Otto, W., Schunemann, N., Wright, M., MacRae, E., Gleave, A. and Laing, W. (2009) Gene expression studies in kiwifruit and gene over-expression in *Arabidopsis* indicates that GDP-L-galactose guanyltransferase is a major control point of vitamin C biosynthesis. J. Exp. Bot. **60**, 765-778

5 Blaschke, K., Ebata, K. T., Karimi, M. M., Zepeda-Martinez, J. A., Goyal, P., Mahapatra, S., Tam, A., Laird, D. J., Hirst, M., Rao, A., Lorincz, M. C. and Ramalho-Santos, M. (2013) Vitamin C induces Tet-dependent DNA demethylation and a blastocyst-like state in ES cells. Nature **500**, 222-226