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Risk Assessment Reference: Regulatory sequences in GM plants

Introduction

Regulatory sequences are small portions of non-coding nucleic acid molecules that often reside in the 5' and 3' untranslated regions (UTRs) of genes within all organisms and viruses. Their role is to facilitate or modulate gene expression at the DNA (transcriptional) or RNA (translational) level, depending if they are located directly in the DNA strand, e.g. promoters, enhancers, introns, insulators, silencers or terminators; or added to the RNA molecule during post-transcriptional modification, e.g. 5'-cap or polyadenylation tails (Vaughn et al., 2012; Biłas et al., 2016).

Promoters allow the RNA polymerase to bind and initiate correct transcription (Picot et al., 2010), and they are able to target gene expression to specific tissues, precise development time-points, or make the expression constitutive, i.e. lead to constant expression levels in most tissues. Some promoters are sensitive to a particular substance, environmental condition or disease, and gene expression is induced or inhibited when these triggers are present. Terminators indicate the end of the coding regions, and polyadenylation signals are necessary for translation of mature mRNA. Other regulatory sequences may also be present, such as enhancers or silencers that increase or decrease the expression pattern of a given gene by binding a transcription factor; leader sequences (5' UTRs) and transit peptide coding sequences that may contribute to protein translation and localisation; or operators that bind repressor proteins to block the RNA polymerase and prevent expression.

Risk considerations

When a GM plant is made by introducing one or more genes, DNA regulatory sequences, also known as *cis* regulatory elements, are used in the DNA cassettes to enable the expression of the inserted transgenes. For the purpose of this Risk Assessment Reference, we will only refer to DNA regulatory sequences from here onwards.

Introduced DNA regulatory sequences are either of plant origin (from the same or other plant), non-plant origin (from any other living organism), or synthetic. The latter might be designed for a specific purpose, such as directing gene expression locally in response to pathogens (Rushton et al., 2002). By using synthetic regulatory sequences, or regulatory sequences from different organisms or tissues, researchers are able to adjust protein expression patterns, such as the location and quantity of the introduced proteins (Dey et al., 2015; Biłas et al., 2016). For example, by using a leaf-specific promoter, the expression of the introduced genes can be targeted just to the leaves of the GM plant (Alotaibi et al., 2018).

To date, GM plants released in Australia contain regulatory sequences from plants such as thale cress, canola, flax, soybean, maize, potato or tobacco, from bacteria such as *Agrobacterium tumefaciens*, or from plant viruses such as the Cauliflower and Tobacco mosaic viruses (OGTR website). Many of these source organisms are widespread and prevalent in the Australian environment, and thus humans and animals are commonly exposed to their regulatory sequences. For example, cauliflower mosaic virus is frequently present in Brassica species that are commonly consumed by people (Saunders et al., 1990).

Some of the above mentioned regulatory sequences are derived from organisms that are associated with allergic reactions in susceptible individuals, toxic responses in people and animals or disease in people, animals or plants. Toxicity responses are generally dose-dependent, and could be associated with overexpression of a gene regulated by a constitutive promoter (Xiong et al., 2019). Since DNA regulatory sequences are non-coding, they cannot encode proteins that are pathogenic or allergenic, and DNA itself does not directly cause disease or allergic reactions (Bannon, 2004). Furthermore, dietary DNA has no toxicity (Toxicology, 2003), and no credible reports have been found indicating that regulatory sequences in GM plants could cause adverse effects when ingested.

Vertical or horizontal transfer of introduced regulatory sequences to other organisms could potentially alter their gene expression in unpredictable ways. However, introduced regulatory sequences operate in the same manner as endogenous sequences, and the majority are already present in the environment and available for transfer via natural mechanisms. Consequently, the impact of transfer of introduced regulatory elements from GM plants is not likely to be greater than that arising from the transfer of endogenous regulatory elements from GM or non-GM plants. As there is no difference between these events, this does not represent a novel adverse outcome as a result of the genetic modification.

Conclusion

Regulatory DNA sequences have no direct pathogenic, toxic or carcinogenic properties, and cannot cause disease. Furthermore, there is no difference in potential impact between vertical or horizontal transfer of introduced *versus* endogenous regulatory sequences to other organisms. Hence, these scenarios do not represent a novel adverse outcome as a result of the genetic modification, and the risks from introduced regulatory elements are considered to be negligible when compared to those from endogenous regulatory sequences from GM or non-GM plants.

References

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