Determination of inauthentic protein glycosylation in transgenic plants

Area of research interest: Novel and non-traditional foods, additives and processes
Study duration: 2007-02-01
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Conducted by: University of Cambridge and Rothamstead Research
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Background

This project aimed to develop robust methods to characterise the glycosylation of proteins for routine analysis of transgenic plants. Standard operating procedures (SOPs) were developed that may be suitable for use by industry and regulatory authorities. The established relationship between protein glycosylation, immunogenicity and allergenicity means that the methods developed could be used to inform the risk assessment of genetically modified (GM) plants.

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Research Approach

Novel methods were developed to identify and characterise N- and O-linked glycans of plant proteins. These methodologies were then developed into SOPs and their performance for routine analysis were evaluated. Methods were tested using plant proteins which are known to be glycosylated, including those from transgenic plants in which the pattern of glycosylation is known to be inauthentic and consequently conferring unexpected allergenicity. These methods allowed the determination of differences in glycosylation patterns between individual glycoproteins as well as between different plants.

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Results

Methods developed and evaluated at the University of Cambridge were successfully transferred to Rothamsted Research where they were applied to transgenic and non-transgenic plants. These included the transgenic pea lines expressing the bean -amylase inhibitor (Prescott et al. 2005) which had caused public concern when they were used in animal feeding studies and found to produce an altered immune response, compared to the native bean ?-amylase inhibitor. The N-glycosylation methods developed at the University of Cambridge were reduced to a Standard Operating Procedure (SOP) at Rothamsted Research suitable for routine use in industry and by regulatory authorities.

Following evaluation of the method at Cambridge with AGP from bread wheat AGP the method was transferred to Rothamsted Research. At Rothamsted the method was applied to an AGP

preparation from bread wheat and the same oligosaccharides identified, verifying the robustness of the method. AGP isolated from durum and einkorn wheats was also analysed and a similar set of oligosaccharides identified. Finally, the method was applied to control and transgenic bread wheat lines, expressing extra copies of AGP. As with N-glycan analysis, the O-linked glycan methods developed worked well but statistical analysis will be required to determine whether the differences are of significant when compared with the range of natural variation.

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Published Papers

1. Tryfona, T., Liang, H.-C., Kotake, T., Kaneko, S., Marsh, J., Ichinose, H., Lovegrove, A., Tsumuraya, Y., Shewry, P.R., Stephens, E. & Dupree, P. (2011) Carbohydrate structural analysis of wheat flour arabinogalactan protein. *Carbohydrate Research*345, 2648-2656, published online doi:10.1016/j.carres.2010.09.018
2. Marsh, J.T., Tryfona, T., Powers, S.J., Stephens, E., Dupree, P., Shewry, P.R. & Lovegrove, A. (2011) Determination of the N-glycosylation patterns of seed proteins: applications to determine authenticity and the substantial equivalence of GM crops. J. Agric. Food Chem., 59 (16), 8779-8788, doi: 10.1021/jf2010854

Research report
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