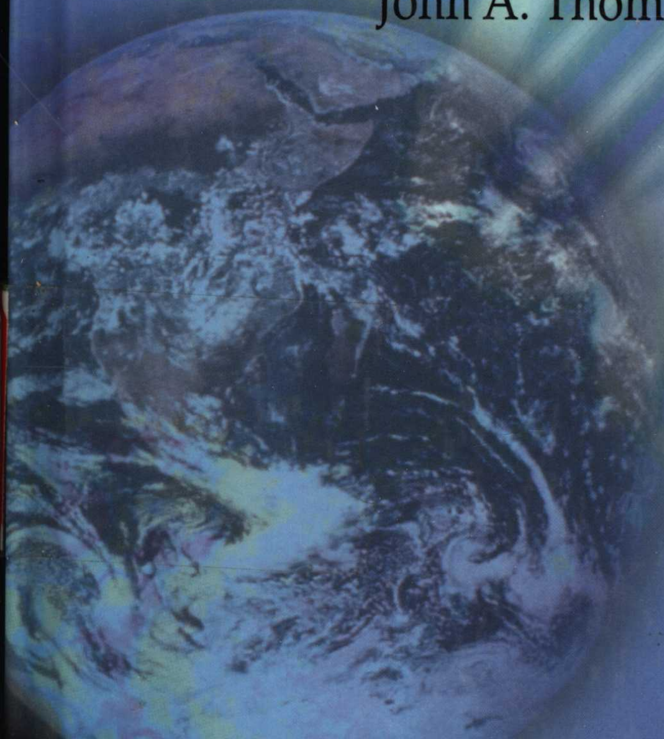
Biotechnology and Safety Assessment

THIRD EDITION

Edited by

John A. Thomas and Roy L. Fuchs



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Preface

The first edition of Biotechnology and Safety Assessment, edited by John A. Thomas and Laurie A. Myers was published in 1993 with its major emphasis on emerging molecular biology techniques used in the production of recombinant DNA-derived drugs as well as describing early protocols designed to ensure their pre-clinical safety and efficacy. Advances in transgenic animal models and safety evaluation approaches to genetically modified (GM) foods were also described.

The Second edition of Biotechnology and Safety Assessment, edited by John A. Thomas in 1999 heralded an expansion of topics in the fields of biotherapeutics and agribiotechnology. It encompassed the latest advances in antisense therapeutics, molecular modification of cytokines, the clinical toxicity of interferons, and the pharmacology of recombinant proteins. This Edition was greatly expanded into areas of agribiotechnology including risk/benefit issues, environmental considerations, food and feed safety assessment and allergens in GM and non-GM foods.

The Third Edition continues to highlight major advances in areas of biotherapeutics and agribiotechnology. The Third Edition is more comprehensive than previous editions and provides important global perspectives on the safety and commercialization of GM crops and newer, more potent therapeutics agents. Biotechnology and Safety Assessment, 3rd edition is edited by John A. Thomas and Roy L. Fuchs and contains chapters written by internationally recognized experts in the fields of molecular genetics, nutrition, food science and safety/risk assessment. It contains a wide spectrum of topics yet integrates them into an overall approach involving safety testing, regulatory oversight and post-marketing surveillance. Many topics are especially important to the toxicologist, the pharmacologist, the nutritionist and those responsible for assessing risk/benefit and environmental impacts and the safety of GM pharmaceuticals, microbial products and plant products.

Through recent advances in agribiotechnology there is a transition from crop genetically modified for improved insect, weed and disease control to crops with enhanced nutritional properties such as vitamin and other micronutrients or safer foods with decreases allergenic concerns.

There is truly a revolution in food technology and one that will lead to helping feed the burgeoning world population in the 21^{st} century. Finally, chapters are specifically devoted to the pre-clinical safety of GM microorganisms used in food processing and fermentation, and to immunotoxicological testing protocols for cytokines and other therapeutic proteins. The environment, non-target species, and risk/benefit topics are covered in significant depth to make this Third Edition a valuable resource for the corporate technical library and for the medical center library. It will also be very beneficial to the biomedical scientist's book shelf whether their field is molecular genetics, agronomy, microbiology, nutrition or a healthcare provider seeking to better understand the rapid progress being made in biotechnology.

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Chapter 1

Using Plant Biotechnology to Reduce Allergens in Food: Status and Future Potential

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Food allergic reactions affect 6–8% of children and 1–2% of the adult population. The incidence of IgE-mediated reactions to specific food crops is increasing, particularly in developed countries, likely owing to increased levels of protein consumption. Many allergic reactions are to foods of plant origin, including peanuts, soy, wheat, and tree nuts. Allergic reactions are typically elicited by a defined subset of proteins that are found in abundance in the food. The increased prevalence of allergic reactions coupled with the sometime severe clinical symptoms has led many scientists to explore methods of reducing the allergenicity of some crops. This chapter explores the potential to reduce allergenicity of plants used as food crops by both traditional breeding practices and genetic engineering methods.

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INTRODUCTION

A mere 20 years ago the improvement of crop productivity and heartiness was a trial-and-error process; sometimes it took years to determine whether a desired trait was stable in a new hybrid. This process depended on the existence of natural variation in the plants of interest or on our ability to create variability by chemical or irradiation mutagenesis coupled with our ability to identify specific phenotypic characteristics that might improve a plant's production potential. Once desirable phenotypic qualities had been identified, the laborious task of crossing and back crossing plants was started in the hope of moving whatever genetic material was responsible for this phenotype into the new hybrid line, without introducing any undesirable traits. There are obvious limitations to this approach, primarily the requirement that there be a naturally occurring variant with the desired phenotypic trait or the ability to create such variation via mutagenesis or other methods, and the time-consuming and labor-intensive process of hybrid production. Even with these limitations, crop scientists and geneticists were able to improve most crop yields severalfold to feed an ever-expanding world population.

With the advent of molecular biology and biotechnology it became possible not only to identify a desirable phenotypic trait but also to identify the precise genetic material responsible for that genetic trait. Recombinant DNA and plant transformation techniques have made it possible to alter the composition of individual plant components (lipids, carbohydrates, proteins) beyond what is possible through traditional breeding practices. The thrust of most plant biotechnology programs has been to enhance or reduce the level of specific components naturally found in the plant or to introduce a component not naturally found in the plant. One example of a naturally occurring component in a plant that has been increased in a biotechnology-engineered crop is the starch content in potatoes. Starch consists of three components in varying amounts depending on the plant source: the large linear molecules of amylose, complex branched amylopectin, and a smaller size amylose (Baba and Arai, 1984). As might be expected, the starch biosynthetic pathway is complicated, with many enzymes involved in producing the final product. However, the product of the ADPG pyrophosphorylase gene (reviewed by Smith *et al.*, 1995) appears to control the overall flux through the starch biosynthetic pathway. In this example, Stark *et al.* (1992) utilized the non-feedback-inhibited ADPG pyrophosphorylase gene from *E. coli* to increase the starch content of potatoes.

One example of a biotechnology-engineered crop distinguished by a component that is not naturally found in a plant is "golden rice." This biotechnology-derived rice line was developed to combat vitamin A deficiency, the leading cause of severe visual impairment and blindness among children in developing countries. In this rice line, genes encoding proteins necessary for the production of β-carotene, the precursor of vitamin A, were introduced into the genome. Successful integration and functioning of the genes resulted in rice plants that produced yellow-tinted kernels with the intensity of color indicating the amount of β-carotene present (Friedrich, 1999). Genes were also introduced to increase the level and bioavailability of iron, another important nutrient.

In addition to investigations aimed at improving the nutritional quality of food crops, a large body of work has been targeted at improving resistance to insect predation. Gene transfer work utilizing the bacterial (*Bacillus thuringiensis*) crystal protein (Bt-Cry) produced genes with resistance to a range of lepidopteran insects. Cauliflower, corn, and tomato varieties have been successfully transformed with vectors expressing insecticidal Bt-Cry proteins with no significant changes in key nutrients, overall composition of unknown metabolites, or *N*-glycans.

It is important to recognize that modifying plant genomes introduces the possibility of altering the allergenic potential of foods whether that change is brought about by classic plant breeding practices or by directed gene approaches. This can happen by increasing the allergenic potential of resident allergenic proteins or by introducing completely novel proteins that have characteristics of food allergens. Methods and safety assessment approaches have been developed and applied to address these concerns (Taylor and Hefle, Chapter 11, this volume). However, biotechnology can also be used to directly decrease the levels of known allergens or their allergenicity. With this in mind, this chapter focuses on some of the approaches being taken to reduce the allergenic potential of foods derived from major crops.

CHARACTERISTICS OF FOOD ALLERGENS

Before reviewing the different approaches to reducing the allergenic potential of foods, it is important to mention the components of foods that are classified as allergens. There are about 26 major allergens identified for about 17 different food items (IUIS Allergen Nomenclature Subcommittee, 1997). From biochemical analysis of this limited number of allergens, certain characteristics shared by most but not necessarily all can be identified. For example, food allergens are typically low molecular weight glycosylated proteins that are relatively abundant in a food source. In addition, they have acidic isoelectric points, as well as multiple, linear IgE binding epitopes, and are resistant to denaturation and digestion (Stanley and Bannon, 1999). These characteristics are purported to be important to the