Gene 515 (2013) 241–248

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Review

Evaluation of designer crops for biosafety—A scientist's perspective

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ARTICLE INFO

Article history:
Accepted 4 December 2012
Available online 22 December 2012

Keywords:
Transgenics
Phenomics
Transcriptomics
Proteomics
Metabolomics
Commercialization

ABSTRACT

With the advent of transgenic technology, it has become possible to mobilize and express foreign genes into plants and to design crop varieties with better agronomic attributes and adaptability to challenging environmental conditions. Recent advances in transgenic technology have led to concerns about safety of transgenic crops to human and animal health and environment. Biosafety focuses on preventing, minimizing and eliminating risks associated with the research, production, and use of transgenic crops. Food biosafety involves studies of substantial equivalence related to compositional analysis, toxicity and allergenicity. Environmental biosafety involves glasshouse and field trials and study of unintended effects on non-target organisms. Transgenics are characterized at phenotypic and molecular levels for understanding the location of transgene insertion site, ploidy level, copy number, integrated vector sequences, protein expression and stability of the transgene. Various techniques employed for transgene characterization include flow cytometry, southern, northern and western analyses, real-time (qRT) PCR, competitive PCR, FISH, fiber-FISH, DNA micro-arrays, mRNA profiling, 2DE-MS, iTRAQ, FT-MS, NMR, GC–MS, CE–MS and biosensor-based approaches. Evaluation of transgene expression involves the application of integrated phenomics, transcriptomics, proteomics and metabolomics approaches. However, the relevance and application of these approaches may vary in different cases. The elaborate analysis of transgenic crops will facilitate the safety assessment and commercialization of transgenics and lead to global food security for the future.

Contents

Abbreviations: PCR, polymerase chain reaction; qRT-PCR, quantitative real-time PCR; FISH, fluorescence in situ hybridization; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; mRNA, messenger RNA; ELISA, enzyme linked immunosorbent assay; TAIL-PCR, thermal asymmetric interlaced PCR; ORF, open reading frame; RFLP, restriction fragment length polymorphism; 2DE/MS, two-dimensional electrophoresis mass spectrometry; FT/MS, Fourier-transform ion cyclotron mass spectrometry; GC/MS, gas chromatography mass spectrometry; CE/MS, capillary electrophoresis mass spectrometry; LC/MS, liquid chromatography mass spectrometry; NMR, nuclear magnetic resonance; ^{1}H NMR, proton nuclear magnetic resonance; T-DNA, transfer-DNA; AFLP, amplified fragment length polymorphism; iTRAQ, isobaric tags for relative and absolute quantification; PAT, phosphinothricin acetyl transferase; A, adenosine; T, thymine; GEO, genetically engineered organisms; GM, genetically modified.

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0378-1119/$ – see front matter
http://dx.doi.org/10.1016/j.gene.2012.12.029

1. Introduction

With the ever-increasing threat of global population, there has been an urgent need for enhancement of productivity in crop plants by infusion of new genetic variability and improvement of the nutritional and industrial utility of the crop species. Effective utilization of genetic diversity remains a pivotal factor in designing crop varieties with better agronomic attributes and adaptability to challenging environmental conditions. Biotechnology is emerging as one of the most innovative tool in life sciences and is influencing almost every aspect of human life. The feasibility of mobilizing and expressing foreign genes into plants has opened up a new era of genetically engineered (transgenic) crops. With limited natural resources available to improve agricultural production, genetically engineered crops provide a promising alternative for improving and enhancing crop productivity. Last few years have witnessed a remarkable progress in the production and cultivation of transgenic crops.

Organisms whose genomes have been altered by the insertion of a foreign gene or genes from another species or unrelated organism are known as transgenics or genetically engineered organisms (GEOs). They have detectable fragments of expected size and acceptable values of foreign/ transgene protein. They carry the transgene which when integrated and expressed stably and properly, confers either a new trait to the organism or enhances or suppresses an already existing trait. Intensive research over the past few decades has resulted in the development of effective gene transfer procedures with subsequent recovery of genetically modified plants. Today's commercialized transgenic plants have been produced using *Agrobacterium*-mediated transformation or gene gun-mediated transformation/ microprojectile bombardment. Novel techniques allow precise manipulation of transgene incorporation and can help to secure stable expression of the transgenics in a wide range of environmental conditions (Tsaftaris et al., 2000).

Insertion of foreign gene into plants has made them capable of defending against natural stresses (biotic and abiotic) with enhanced survival, persistence and competitive capabilities, producing biofuels, vaccines and antibodies and better quality products and novel compounds of commercial value and improving the nutritional quality of food products. To date, various transgenes have been successfully introduced into the nuclear genomes of various plant species. Major crops where transgenics are commercially available include rice, soybean, maize, cotton, canola, potato, cassava, squash, papaya, groundnut, oilseeds and various vegetables and fruits (Asif et al., 2011; Chakraborty et al., 2010; Hutchison et al., 2010; Llorente et al., 2010; Mendoza et al., 2008; Motoyama et al., 2010). Significant improvements in the commercially available crops including herbicide resistance, insect and virus resistance and nutritional quality enhancement have been achieved (Table 1). The role of genetic engineering in generating transgenic lines/cultivars of different crops with improved nutritional quality, biofuel production, enhanced production of vaccines and antibodies, herbicides, and increased resistance to biotic and abiotic stresses has also received attention (Ahmad et al., 2012). The world's leading producers of transgenic crops are USA, Brazil, Argentina, India, China, Paraguay and South Africa.

Table 1
Commercialized transgenic food crops and the significant improvements achieved.

Transgenic crops	Significant improvements
Corn, soybean, rice, corn and sugar beet	Herbicide resistance
Corn, rice, tomato and potato	Insect Pest resistance
Papaya, squash and potato	Virus resistance
Tomato and melon	Delayed ripening, increased shelf-life
Canola and soybean	Improved oil quality
Canola and corn	Male sterility

2. Biosafety concerns

Rapid advances in the development and commercialization of transgenic crops have led to considerable apprehensions and concerns about the safety of transgenic-derived products for human, animal health and environment. Transgenics are also a subject of a strong hostility especially in Europe as they are perceived as hardly useful, non-natural and risky. Transgenic crops are not considered suitable for agriculture in the European Union due to specific scientific and environmental safety reasons. They are considered to have high potential risks and fewer benefits. They comprise biohazards like creation of virulent microorganisms and other life forms that may endanger human and environment. They also comprise potential dangers and risks for farmers and for biodiversity since the landscape and regional dimension of vertical gene flow make almost impossible the coexistence of transgenic crops with organic farming in Europe. Food derived from transgenic crops also poses human health concerns. However, recently the approval of transgenic 'Amflora' potato by the European Commission in 2010 has signaled a fresh approach to genetically engineered organisms in Europe.

The major risks perceived because of the introduction of transgenic crops include i) increased weediness, ii) transgene flow into different cultivars/related species/unrelated organisms, iii) possibility of development of new viruses with wider host range, iv) pest resistance, v) unintended delirious effects on non-target species including biological control agents, v) safety of foods derived from transgenics, vii) feed safety, viii) fate of protein in soil, and other unforeseen effects. These risks may not be universal but may vary depending upon the transgenic lines. This has led to debates over the need for rigorous risk assessments including the long-term effects before permitting the release of any transgenic-derived food products. Mulvaney et al. (2011) have reviewed the economic benefits and risks to marketability of transgenics.

The safe application of biotechnology in agriculture for minimizing risks to the environment and human health from the handling and transfer of transgenics is termed as biosafety. The concerns over ecological and food biosafety have escalated beyond scientific rationality. To ensure the biosafety of transgenic crops, there is a need for a comprehensive analysis of transgenic plants and their progenies, and their complex interactions that can influence their performance. It is imperative to build scientific expertise and resources for assessment of possible risks before commercialization of GEOs are contemplated. The Biosafety Laws establish safety standards and enforcement mechanisms on the construction, cultivation, production, handling, transportation, transfer, import, export, storage, research, marketing, consumption, release into the environment and disposal of transgenics and their derivatives for protection of human and animal health and the environment. The following sections provide an overview of the concerns of food and environment safety and the approaches to analyze them, which have been presented in a form of artwork (Fig. 1).

2.1. Food safety

Food biosafety research focuses on the assessment of novel foods produced from transgenic crops which is performed by comparing them with the conventional analog with an established history of safe use in a study called substantial equivalence, which is done