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Review Article

Perspectives on genetically modified crops and food detection

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ABSTRACT

Genetically modified (GM) crops are a major product of the global food industry. From 1996 to 2014, 357 GM crops were approved and the global value of the GM crop market reached 35% of the global commercial seed market in 2014. However, the rapid growth of the GM crop-based industry has also created controversies in many regions, including the European Union, Egypt, and Taiwan. The effective detection and regulation of GM crops/foods are necessary to reduce the impact of these controversies. In this review, the status of GM crops and the technology for their detection are discussed. As the primary gap in GM crop regulation exists in the application of detection technology to field regulation, efforts should be made to develop an integrated, standardized, and high-throughput GM crop detection system. We propose the development of an integrated GM crop detection system, to be used in combination with a standardized international database, a decision support system, high-throughput DNA analysis, and automated sample processing. By integrating these technologies, we hope that the proposed GM crop detection system will provide a method to facilitate comprehensive GM crop regulation.

1. Introduction

Genetically modified (GM) crops are a dominant agricultural food product worldwide owing to their superior productivity. From 1996 to 2014, 357 GM crops have been approved globally. The global value of the GM crop market was 15.7 billion US$ in 2014, representing 35% of the global commercial seed market [1]. Rapid growth of the GM crop industry also created controversies in many regions, including the European Union [2], Egypt [3,4], Japan [5], Korea [6], Brazil [7], and Taiwan [8]. To mitigate these controversies, effective regulation based on comprehensive GM crop detection is essential. DNA-based methods such as real-time quantitative PCR (qPCR) have been successfully applied to GM crop detection for the past two decades. However, the continued rapid development of new GM crop events is overwhelming the processing capacity of conventional methods. In addition, the efficacy of GM crop regulation has deteriorated further, due to the release of unauthorized GM crops/foods into the food chain [9]. To meet these challenges, it is necessary to develop a high-efficiency GM crop detection infrastructure.

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2. Classification of GM crops and levels of DNA detection

The past few decades have seen significant advances in plant gene engineering. The methods for the transgenic manipulation of GM crops have also evolved, with major breakthroughs in both technology and theory. Today, GM crops can be classified into four generations according to the structure and strategy used to construct their transgenes. Therefore, the detection of GM crops/foods requires a dedicated strategy. GM crops/foods can be identified via several types of biomolecules such as specific proteins, RNA, DNA, and metabolites. Among these targets, DNA is the only molecule with advantages of being stable, abundant, and easily to amplify. Thus, detection of specific DNA sequences, especially using a PCR-based approach, is still the most effective strategy. In brief, there are four generations of GM crops and three major levels of detection.

(1) Four generations/classes of GM crops

(a) The first generation/class: single trait

Most commercial GM crops today either are of the first generation or its stacked (second generation) [9]. Most first-generation GM crops contain common transgene elements such as the cauliflower mosaic virus (CaMV), 35S promoter (*CaMV35S*-P), aminoglycoside 3′-phosphotransferase gene (*npt*II), phosphinothricin acetyltransferase gene (*pat/bar*), 5-enolpyruvylshikimate 3-phosphate (CP4-*epsp*) gene, nopaline synthase promoter (*nos*-P), and terminator (*nos*-T). In effect, because of the limited variation in high-performance transgene elements, ~90% of commercial GM crops contain one or more of the six transgene elements listed above [10].

(b) The second generation/class: stacked traits

Second-generation GM crops are usually hybrid crosses between commercialized first-generation GM crops {e.g., 59122 × MIR604 maize (DAS-59122-7 × SYN-IR604-5) [9]}. Owing to their lower developing costs, the importance and prevalence of second-generation GM crops are increasing. However, two major detection problems arose with stacked trait GM crops/foods: (1) in-depth gene analysis may require the ability to discriminate between stacked trait GM crops and *unintended stacked trait* GM crops, which might be produced via cross-pollination between two single GM crop events in adjacent fields and (2) the discrimination of mixed events from single stack traits was only possible by testing single seeds or plants, which prevents the technique from being used on processed GM crop products such as corn flour. The detection of second-generation GM crops is complicated by these problems, which together could pose a major threat to GM crop regulation in the near future.

(c) The third and fourth generations/classes: near-intragenics, intragenics, and cisgenics

The third generation of GM crops is comprised of so-called near-intragenics, or GM crops where the inserted transgenic elements have not been used in other (known) GM crops [9]. Near-intragenics are transgene constructs that originated from the host and have undergone minimal recombination or modification. This makes them more difficult to detect than first- or second-generation GM crops.

True intragenics and cisgenics are to be classified as the fourth generation of GM crops. The transgenic elements of fourth-generation GM crops are genuine host genes. Thus, fourth-generation GM crops/foods cannot be distinguished via their transgenic elements. The only way to identify fourth-generation GM crops/foods is to inspect the specific order and insertion loci of its transgenes.

3. Level of DNA detection

(1) Element-specific

Element-specific PCR methods target individual transgenic elements (such as promoters, genes, or terminators), which may be independent of transgenic traits [9]. Due to the limited variance of transgenic elements, this is a very effective universal GM crop screening strategy, especially in multiplex form. In effect, element-specific PCR methods are the only currently available approaches to effective screening of unauthorized and unintended GM crops. The major drawbacks of element-specific PCR are its limited utility for GM crop quantification and its inability to detect intragenic and cisgenic GM crops. It should be noted that transgenic elements sharing the same name do not necessarily possess identical DNA sequences. Various sequence optimizations and variations introduced during GM crop development may decrease the specificity of element-specific PCR methods [10].

(2) Construct-specific

Construct-specific PCR targets the specific order of transgenic elements [9]. The target sequences of construct-specific PCR are usually comprised of junction(s) of two or more transgenic elements, which do not exist naturally in organisms. The resolving power of construct-specific PCR is inferior to that of event-specific PCR, because of the many GM crops that share similar transgenic construct configurations. However, the throughput of construct-specific PCR for the screening of GM crops is also constrained by its specificity to constructs but not universal transgenic elements. Thus, despite the fact that the discriminatory ability of construct-specific PCR is higher than that of element-specific PCR, construct-specific methods used in routine GM crop detection are rare. The method is simply too general for use in the identification of GM crops while being an inefficient screening method.

(3) Event-specific

As most plant transformation methods (such as *Agrobacterium* or Biolistic) used today are based on the random insertion of transgenic DNA, chimeric sequences comprised of host DNA and transgenic construct border sequences are present in every trait of GM crops [9]. Event-specific PCR