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Research review paper

Molecular characterization of genetically-modified crops: Challenges and strategies

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ABSTRACT

Molecular characterization lays a foundation for safety assessment and subsequent monitoring of genetically modified (GM) crops. Due to the target-specific nature, conventional polymerase chain reaction (PCR)-based methods cannot comprehensively detect unintended gene insertions, let alone unknown GM events. As more and more new developed GM crops including new plant breeding technology (NPBT) generated crops are in the pipeline for commercialization, alternative -omics approaches, particularly next generation sequencing, have been developed for molecular characterization of authorized or unauthorized GM (UGM) crops. This review summarizes first those methods, addresses their challenges, and discusses possible strategies for molecular characterization of engineered crops generated by NPBT, highlighting needs for a global information-sharing database and cost-effective, accurate and comprehensive molecular characterization approaches.

Contents

1. Introduction

Molecular characterization of genetically modified (GM) crops provides structural and expressional information of the insert(s) and stability information of the intended trait(s) (EFSA, 2011a, EFSA, 2012a). Molecular characteristics of GM crops generally include genomic features (such as insertion site, flanking sequence and copy number),

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transcriptomic features, proteomic features, and metabolomic features (Fig. 1), and information of these features is fundamental for the research & development, safety assessment, detection, and monitoring of GM crops. Based on molecular characteristics, both intended and unintended effects of transgene(s) can be readily identified (EFSA, 2012a, Schnell et al., 2015), facilitating significantly subsequent efforts for risk assessment of potential effects of GM crops and their derived products on food/feed quality and safety (Schnell et al., 2015).

The principle of substantial equivalent is one of the key principles of risk assessment for GM crops. To see if a GM crop is substantially equivalent to its non-GM recipient, various omics-based systems biology approaches are applied to compare their molecular characteristics (Heinemann et al., 2011). Most of current molecular characterization methods for GM crops are based on PCR based approaches (Fraiture et al., 2015b; Arulandhu et al.; 2016). Although transcriptomic (Coll et al., 2010; Li et al., 2016), proteomic (Gong and Wang, 2013), and metabolomics (Clarke et al., 2013; Simo et al., 2014) characterization have been applied to some GM crops, comprehensive system biology analyses at all levels (DNA, RNA, protein and metabolite) on a GM crop event are still very rare (Ricroch et al., 2011). Since more and more GM crops as well as novel engineered crops derived from new plant breeding technology (NPBT) are in the pipeline to be commercialized and released to environment and/or market in the near future (James, 2016; Parisi et al., 2016), it creates great challenges for molecular characterization. To provide detailed molecular characteristics of both authorized and unauthorized GM (UGM) crops to regulators, retailers, and consumers, development of accurate, comprehensive, and cost-effective molecular characterization methods are urgently needed. In the following we first address the technical aspects of molecular characterization of GM crops, focusing mainly on DNA based technologies; then we will highlight the main challenges and also discuss potential coping strategies.

2. Current methods for molecular characterization of GM crops

2.1. PCR-based methods for characterization of insertion sites and unknown flanking regions

Identifying the event-specific insertion sites upstream or downstream of an exogenous insertion in a GM crop provides direct evidence of the unknown flanking regions and conclusive evidence of the identity of the given GM crop. For this purpose, many PCR-based genome walking methods, such as thermal asymmetric interlaced PCR (TAIL-PCR) (Liu and Whittier, 1995), ligation mediated PCR (LM-PCR) (Mueller and Wold, 1991), and inverse PCR (IPCR) (Ochman et al., 1988), have been originally adopted. Later on, more and more modified or improved PCR-based methods, high throughput or high efficiency Tail PCR (Liu and Chen, 2007; Singer and Burke, 2003), and adaptor mediated PCR (Huang et al., 2007) were developed for routine molecular characterization with high throughput and/or high efficiency. These PCR-based methods all rely on known sequence information of the exogenous insertion, and no single method can be applied universally. Each method possesses its own characteristics (Table 1), and combinations of different methods are often adopted to increase efficiency (Yang et al., 2007). Because PCR-based methods cannot detect undocumented molecular characteristics of GM crops, in many cases, T-DNA insertion information is often underestimated (Yang et al., 2013a). Taking the commercialized soybean event GTS40-3-2 as an example, initially PCR-based methods identified only one inserted copy of the expression cassette of EPSPS gene (5-enolpyruvylshikimate 3-phosphate synthase) in the host's genome (Padgette et al., 1995). However, further studies revealed two additional unintended partial insertions of CP4 EPSPS (72- and 250-base pair, respectively) (Product Safety Center, 2000) and unintended DNA rearrangements at the 3′-NOS junction, causing the molecular characterization of GTS40-3-2 to be amended several times (Windels et al., 2001). Other examples involve GM insect resistant rice TT51-1 and T1c-19 that were developed in China. Initial PCR-based gene walking approaches identified one full insertion cassette each on the Chromosome 10 of TT51-1 (Cao et al., 2011) and the chromosome 11 of T1C-19 (Tang et al., 2006), respectively. Further whole genome next generation sequencing (NGS) approach revealed an additional full insertion cassette each on the chromosome 4 of TT51-1 and on the chromosome 4 of T1c-19, respectively (Yang et al., 2013a). Therefore, more attention should be paid to efforts making PCR-based methods also effective in characterizing unknown GM crops fully taking the advantage of bioinformatics tools and other related approaches.

2.2. Next generation sequencing based methods for comprehensive characterization of insertion sites and unknown flanking regions

As evidenced in several recent reviews, the combinations of abovementioned PCR based approaches with NGS appear to be more accurate and more comprehensive for molecular characterization of GM (or UGM) crops (Bodi et al., 2013; Mertes et al., 2011; Arulandhu et al., 2016). The process involves an initial enrichment of unknown adjacent sequences of known GM elements before NGS, using target-specific primers (not semi-random or random primers). Therefore, not all PCR-based methods can be effectively coupled with NGS to characterize GM and particularly UGM crops universally (Arulandhu et al., 2016). So far, none of the available enrichment methods can enrich long enough DNA fragments down- or up-stream of a known insert in a very sensitive manner, and none of them has been demonstrated to be effective in

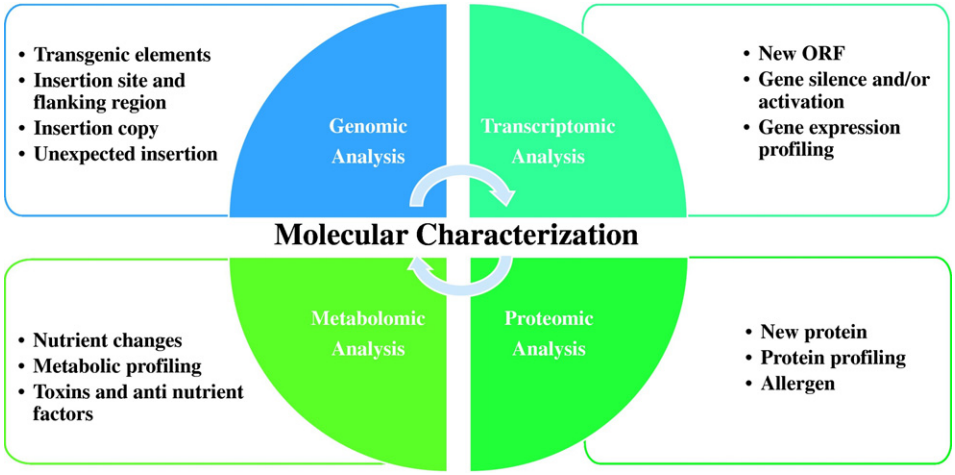

Fig. 1. Molecular characterization of genetically modified crops at genomic, transcriptomic, proteomic, and metabolomic levels.