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## Review

## Biotech rice: Current developments and future detection challenges in food and feed chain

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## ABSTRACT

**Background:** To improve agricultural practices and the food/feed security, plant breeding techniques were developed, including transgenesis commonly using *Agrobacterium tumefaciens* or biolistic technologies. To guarantee the traceability of GMO in food/feed chain and the consumer's freedom of choice, regulatory frameworks were established in many countries around the world, such as in Europe. Their implementations, including detection systems usually based on qPCR, are becoming complex and expensive regarding the number of analysis to perform. Moreover, the dispersion of publicly available information about developed GMO prevents to accurately estimate the efficiency of the standard detection system applied to unauthorized GMO.

**Scope and approach:** To illustrate this problem, the case of rice, one of the leading staple crops, was investigated. An overview of worldwide developed biotech rice generated by transgenesis was thus conducted, based on 1067 peer-reviewed publications, and analysed regarding *inter alia* their expressed genes of interest and the corresponding traits, their transformation processes and the elements composing their transgenic cassettes. From this work, the power and weakness of the standard detection system, notably used by the European enforcement laboratories, are evaluated. To strengthen this system, especially with unauthorized GMO, additional strategies are suggested. Moreover, given the growing interest for biotech rice produced by new plant breeding techniques, related challenges for their detection are discussed.

**Key findings and conclusions:** According to all collected information, suitable detection strategies, combining qPCR to additional technologies (e.g., DNA walking and NGS), are proposed to cover most of inventoried biotech rice. The present approach, including the data centralization to subsequently suggest appropriated detection strategies, can be extended to biotech events from different species.

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## 1. Introduction

To cope with the challenge of increasing the crop production, the evolution of current agricultural practices was envisaged via the support of conventional breeding methods by contemporary approaches. Therefore, various strategies have been used to develop biotech crops, such as transgenic crops, also named genetically

modified (GM) crops or genetically engineered crops, opening new possibilities to reach the expected crop nutritional necessity and to ensure food security (Ahmad et al., 2012; He, Xia, Peng, & Lumpkin, 2014). As observed for several crops, genetic engineering had *inter alia* been applied to rice (*Oryza sativa*), currently one of the most important cereal crops that is cultivated in many countries through the world. For more than 3.5 billion people, rice is a staple food providing more than 20% of their daily calories intake, especially in developing countries. In addition, this crop is also intended to feed animals. In 2014, the annual production of paddy rice was estimated at 741.3 million tons (corresponding to 494.4 million tons of

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milled rice) where the majority was grown in Asia (674.4 million tons; 91%), mainly in China (208.1 million tons), India (155.5 million tons), Indonesia (70.6 million tons), Bangladesh (52.4 million tons) and Vietnam (44.9 million tons). The rest of paddy rice is harvested in Africa (27.6 million tons), South America (24.8 million tons), North and Central America (12.9 million tons), Europe (4.1 million tons) and Oceania (0.9 million tons) (FAO, 2015; Fraiture et al., 2014; Khush, 2013). Therefore, even if no biotech rice is nowadays cultivated worldwide at a commercial scale, its potential significance is clearly obvious in the near future (De Steur et al., 2014).

With the aim to guarantee the traceability on the markets as well as the freedom of choice to the consumers, several genetically modified organism (GMO) labelling systems have been established in several countries with a threshold varying from 0 to 5%. The labelling is either mandatory (e.g., in Australia, Brazil, Chile, China, EU, India, Indonesia, Israel, Japan, Philippines, Russia, Saudi Arabia, South Korea, Taiwan and Thailand) or voluntary (e.g., in Argentina, Canada and USA). On the European Union (EU) market, commercialised food and feed products containing at least 0.9% of EU authorized GMO have to be labelled to guarantee the freedom of choice of the consumers while the zero tolerance is applied on unauthorized GMO (Davison, 2010; Kamle & Ali, 2013). The implementation of these legislations is mainly carried out using real-time PCR (qPCR) technology, allowing to detect, identify and quantify GMO (Fraiture, Herman, Taverniers, De Loose, Deforce et al., 2015). Three main steps are traditionally followed in GMO routine analysis. First, the presence of GMO is detected by screening. It includes simplex or multiplex methods targeting the most common transgenic elements, such as p35S (35S promoter from Cauliflower mosaic virus (CaMV)) and tNOS (nopaline synthase terminator from *Agrobacterium tumefaciens*). In addition, some more discriminative markers are used to reduce the number of subsequent identification. In case of positive signals, the identity and the quantity of GMO are afterwards determined via event-specific methods. If the signals observed during the screening step do not correspond to any of the authorized GM events, the presence of unauthorized GMO is then suspected (Broeders, De Keersmaecker, & Roosens, 2012; Broeders, Papazova, Van den Bulcke, & Roosens, 2012).

Given the ongoing and further expected expansion of GMO in terms of number, diversity and cultivated areas, the implementation of labelling legislations is becoming even more complex. Furthermore, the presence of some GM events could be prohibited or not according to the jurisdiction in reason of the asynchronous authorisations between many countries. Moreover, unlike the present commercialised GM crops which have been mainly developed by American and European companies, more and more GMO produced by national technology centres in developing countries are intended for local consumption. Consequently, these GM crops will probably not be submitted for EU approval. Therefore, the frequency of unauthorized GMO on the EU market is likely to significantly increase due to accidental contamination of non-transgenic raw material and processed food/feed matrices. In addition, unauthorized GMO concerns also GM crops that are currently unknown to the competent authorities (Broeders, De Keersmaecker, et al., 2012; Holst-Jensen et al., 2012; Parisi, Tillie, & Rodríguez-Cerezo, 2016; Stein & Rodríguez-Cerezo, 2009). The complexity of this problematic is particularly well illustrated by GM rice for which no events are nowadays authorized on the EU market. First, the problem of asynchronous approvals has been encountered with products originating from the USA. More precisely, the herbicide tolerant LLRICE601, in 2006, and LLRICE62, in 2007, both produced by Bayer CropScience and exclusively authorized in the USA, were identified in commercial rice matrices. Second, the insect resistant Bt Shanyou 63 and KeFeng-6 were

found in food products originating from China in 2006 and 2010, respectively. These GM rice, produced by Asian research centres, were probably accidentally spread. Besides, the illegal propagation of seeds from field trials as well as their planting by Chinese farmers have been reported (Fraiture et al., 2014; Ruttink et al., 2010; Wang & Johnston, 2007; Wang, Zhu, Lai, & Fu, 2011). Finally, the presence of unknown GM rice was also reported by the RAPID Alert System Database ([http://ec.europa.eu/food/safety/rasff/index\\_en.htm](http://ec.europa.eu/food/safety/rasff/index_en.htm)), allowing notably to notify the detection of unauthorized GMO on the EU market, such as in 2010 and 2011 in products imported from China, contaminated in all likelihood by accident (Fig. 1). Furthermore, still according to the RAPID Alert System Database, practically 50% of analysed food/feed samples between January 2012 and May 2015 contained unauthorized GM rice, including Bt63 and GM Basmati rice, imported from Asia, mainly China. Due to the high level of EU unauthorized GM rice, the EU commission has notably decided to implement "Emergency measures regarding unauthorized genetically modified rice in rice products originating from China and repealing Decision 2008/289/EC" (Commission Implementing Decision no. 2011/884/EU).

The success of the qPCR strategy is directly linked to the availability of information on the targeted sequences, such as from transgenic elements (element-specific markers), association of elements (construct-specific markers) or from junctions between the transgenic cassettes and the plant genomes (event-specific markers). In addition, the quantification of identified GM events requires the availability of Certified Reference Materials (CRM) and taxon-specific methods (Broeders, Papazova et al., 2012; Holst-Jensen et al., 2012). Conversely to EU authorized events for which all these data are accessible in the Compendium of reference methods for GMO analysis, the identification of EU unauthorized events by qPCR strategy could be difficult (Fraiture, Broeders et al., 2015). Indeed, the lack of centralized information about unauthorized transgenic crops is problematic. For instance, for GM rice, only three herbicide tolerant (LLRICE601, LLRICE06 and LLRICE62), four insect resistant (Bt63, Huahui-1, Tarom molaii and GM rice 101096), one fungi resistant (GM rice 101097), six multiple biotic stress resistance (NIA-OS002-9, NIA-OS012-8, NIA-OS004-8, NIA-OS003-1, NIA-OS005-3 and NIA-OS006-4), two abiotic stress resistance (S-C and As-d) and two rice seed edible vaccines against Cedar pollen allergy (7Crp#10 and OsCr11) are currently reported in publicly available GMO databases. Those GM rice lines are mainly described according to the expressed genes of interest and the corresponding traits, the transformation methods used, the transgenic elements contained in the vectors and the related biosafety information (Biosafety Clearing-House (<https://bch.cbd.int/>), Biosafety Scanner (<http://en.biosafetyscanner.org/>), CERA ([http://www.cera-gmc.org/?action=gmc\\_crop\\_database](http://www.cera-gmc.org/?action=gmc_crop_database)), GMO Compass (<http://www.gmo-compass.org/eng/gmo/db/>), GMO register ([http://ec.europa.eu/food/dyna/gm\\_register/index\\_en.cfm](http://ec.europa.eu/food/dyna/gm_register/index_en.cfm))).

Therefore, in this review, after a brief description of the biotech rice history including the transformation technologies used, an overview of transgenic rice events developed through the world was built on the basis of more than 1000 available peer reviewed publications. All data were collected and analysed regarding notably the genes of interest expressed and their origins, the vectors and transgenic elements that composed them, the transformation technologies used and the status of the transgenic rice (laboratory development stage or field trial). In this way, the information on biotech rice centralized in this review can be used to complete publicly available databases as well as to develop and strengthen GMO detection strategies.

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