



Safety aspects of genetically modified crops with abiotic stress tolerance

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Abiotic stress, such as drought, salinity, and temperature extremes, significantly reduce crop yields. Hence, development of abiotic stress-tolerant crops by modern biotechnology may contribute to global food security. Prior to introducing genetically modified crops with abiotic stress tolerance to the market, a food and environmental safety assessment is generally required. Although worldwide harmonised comparative approach is currently provided, risk assessors still face challenges to assess genetically modified crops with abiotic stress-tolerance. Here, we discuss current developments of abiotic stress tolerance as well as issues concerning food and environmental safety assessment of these crops, including current approaches, challenges and future directions.

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Introduction

Abiotic stress, defined as non-living factors negatively affecting living organisms, seriously affects the yield of crops (Bartelmus & Helfand, 1995; Vinocur & Altman, 2005). Crops are constantly challenged by several types of abiotic stress leading to physiological and biochemical changes adversely affecting plant growth and productivity. In practice, this may lead to serious harvest reduction (Boyer, 1982; Bray, Bailey-Serres, & Weretilnyk, 2000; IPCC, 2007; Pachauri, 2007). Drought, salinity and extreme temperatures are most devastating because they can affect almost every aspect of plant physiology and biochemistry, such as nutrient uptake, osmotic and ionic homeostasis, by altering the function and structure of proteins and membranes (Wang, Vinocur, & Altman, 2003). At the same time, it is a big challenge to increase global food production considerably in the decades to come to meet the demand of a rapidly growing world population, but with less land, water and energy, and at the same time preserving natural resources and soil fertility (IFPRI, 2010). Developing abiotic stress-tolerant crops may become a factor to maintain plant growth and productivity. Abiotic stress-tolerant crops can be cultured in areas where other crops cannot easily grow, thereby sustaining and potentially expanding the area for agricultural production.

In recent years, progress has been made in producing crops with abiotic stress-tolerance through either conventional selection and breeding or modern molecular-biological approaches (Pandey *et al.*, 2011). However, broad adoption of genetically modified crops (GM crops), including crops with abiotic stress-tolerance, will depend on adequate safety assessment and related public acceptance. So far, conventionally bred varieties with similar abiotic stress-resistant characteristics generally do not need to go through a pre-market safety assessment. To meet safety concerns in the case of GM crop varieties, many countries have formulated specific regulations to assess the safety of these crops for human and animal consumption and for the environment, prior to market approval (EC, 2013; EFSA, 2011; FAO/WHO, 1996; OECD, 1996, p. 74). In this paper, we will first provide a brief overview of recent developments in abiotic stress-tolerant GM crops. Moreover, we will address current food and environmental safety assessment strategies, discuss the challenges and indicate necessary steps in further developments. Feed

safety is not discussed separately, but under most regulatory regimes food and feed safety will be assessed in a fully integrated way.

Abiotic stress-tolerant crops

In response to abiotic stress, plants adjust themselves at the levels of morphology, phenology, physiology and biochemistry. To obtain abiotic stress-tolerant crop varieties, efforts in recent years have focussed on genes that regulate and/or are involved in metabolic pathways for improving plant tolerance to abiotic stress (Bhatnagar-Mathur, Vadez, & Sharma, 2008). Hussain, Raza, Afzal, and Kayani (2012) provide an overview of engineering plants with osmoprotectant pathways that is subdivided into amino acid-derived osmoprotectants (proline, glycine betaine), sugars and sugar alcohols (trehalose and other carbohydrates) and polyamines. In Hussain, Kayani, and Amjad (2011), an overview is presented on engineered stress tolerance by transcription factors with emphasis on DREB (dehydration responsive binding protein) gene transcription factors. Other approaches are based on enzymes for scavenging active oxygen species, chaperone proteins, such as heat shock proteins, late embryogenesis-abundant proteins, and enzymes modifying membrane lipid saturation (Vinocur & Altman, 2005). Recent reviews on abiotic stress tolerant crops are summarised in Table 1, for those who are interested in this specific topic.

In practice, however, the genetically complex mechanisms of abiotic stress-tolerance make it difficult to introduce abiotic stress-tolerance through genetic modification (Vinocur & Altman, 2005). The first transgenic crop with abiotic stress-tolerance was the drought-tolerant maize (*Zea mays*, MON87460) developed by Monsanto and commercialised in the United States in 2013 (Monsanto, 2013). In addition, several GM crops with abiotic stress-tolerance traits are in the pipeline for commercialisation (Stein & Rodríguez-Cerezo, 2010). For example, genetically modified plant varieties that contain newly introduced osmotin genes in their genome. Osmotin is a small (26 kDa) basic, pathogenesis-related protein that is induced and accumulated as an adaptive response when plants are exposed to abiotic or biotic stress. Subramanyam, Sailaja, Rao, and Lakshmidevi (2011) introduced osmotin into chili pepper, resulting in a yield of peppers in an environment under an abiotic stress of 300 mM NaCl. A transgenic tomato containing the osmotin gene was developed by Goel, Singh, Yadav, Babbar, and Bansal (2010), who showed enhanced tolerance to salt and drought stress compared to the wild type. A drought tolerant rice line containing an osmotin gene is expected to be commercialised in India in 2015 (Stein & Rodríguez-Cerezo, 2010).

More experimental approaches include the introduction of a gene coding for isopentenyltransferase under regulation of a cold-inducible promoter, for increased drought and salt tolerant crops, also at lower temperatures. Isopentenyltransferase is a critical enzyme in the cytokinin

biosynthetic pathway. It is experimentally introduced in cotton (Liu et al., 2012), sugarcane (Belintani, Guerzoni, Moreira, & Vieira, 2012) and peanut (Qin et al., 2011). The induction of glycine betaine synthesis using a *codA* gene is tested in tomato (Goel et al., 2011). Here, mature leaves of transformants revealed higher levels of relative water-, chlorophyll-, and proline content compared to wild-type plants under salt and water stresses, suggesting induction of glycine betaine synthesis and increased tolerance to salt and water stress. Similarly, a gene encoding betaine aldehyde dehydrogenase has been introduced in alfalfa (Liu et al., 2011), and genes encoding trehalose-6-phosphate synthase and delta-pyrroline-5-carboxylase synthase have been introduced in rice conferring increased salt and drought tolerance (Li, Zang, Deng, & Wang, 2011).

Also more general stress-related enzymes have been introduced with the aim to obtain abiotic stress-tolerant plants, for example the introduction of tau-class glutathione-S-transferase in tobacco (Jha, Sharma, & Mishra, 2011), and arginine decarboxylase in tomato (Groppa & Benavides, 2008) and tobacco (Wang, Zhang, Liu, & Li, 2011). For commercial use of these GM plant varieties, a food- and environmental safety assessment will be required. Below, we will evaluate which specific aspects of abiotic stress-tolerant GM plant varieties need to be taken into account in the respective pre-market safety assessments.

Food safety assessment of abiotic stress-tolerant plants

Global consensus has been reached on the basic scientific strategy to assess GM plants and derived products thereof (FAO/WHO, 1996). This strategy has now been incorporated in regulations in many countries (Canadian Government, 2012, chap. 870; EFSA, 2011; FDA, 1992, 1997) and is basically a comparative safety assessment (Kok, Keijer, Kleter, & Kuiper, 2008). The comparative character of the food and feed safety assessment indicates that GM plants as such will not be assessed for their safety, but that the safety of comparable conventional varieties will be used as a baseline for the safety assessment of the newly developed plant variety. The outcome of the assessment will thus not be that the GM plant is safe or not, but the result will be formulated in terms of 'as safe as' comparable conventional varieties that we consider as safe (EC, 2013; EFSA, 2011; FAO/WHO, 1996).

As a result of this global consensus the safety assessment of GM plants is largely harmonised, although differences on details can still be observed. Usually, the safety assessment of GM plants will comprise the delivery of data by the applicant on i) a detailed (molecular) characterisation of the genetic modification, including details on the intended effects, ii) a phenotypic and agronomic comparison of the GM plant with close comparators, and iii) a comparative compositional analysis of key compounds, both nutrients and anti-nutrients (EC, 2013; EFSA, 2011;

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