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REVIEW ARTICLE

An overview of the last 10 years of genetically engineered crop safety research

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Abstract

The technology to produce genetically engineered (GE) plants is celebrating its 30th anniversary and one of the major achievements has been the development of GE crops. The safety of GE crops is crucial for their adoption and has been the object of intense research work often ignored in the public debate. We have reviewed the scientific literature on GE crop safety during the last 10 years, built a classified and manageable list of scientific papers, and analyzed the distribution and composition of the published literature. We selected original research papers, reviews, relevant opinions and reports addressing all the major issues that emerged in the debate on GE crops, trying to catch the scientific consensus that has matured since GE plants became widely cultivated worldwide. The scientific research conducted so far has not detected any significant hazards directly connected with the use of GE crops; however, the debate is still intense. An improvement in the efficacy of scientific communication could have a significant impact on the future of agricultural GE. Our collection of scientific records is available to researchers, communicators and teachers at all levels to help create an informed, balanced public perception on the important issue of GE use in agriculture.

Introduction

Global food production must face several challenges such as climate change, population growth, and competition for arable lands. Healthy foods have to be produced with reduced environmental impact and with less input from non-renewable resources. Genetically engineered (GE) crops could be an important tool in this scenario, but their release into the environment and their use as food and feed has raised concerns, especially in the European Union (EU) that has adopted a more stringent regulatory framework compared to other countries (Jaffe, 2004).

The safety of GE crops is crucial for their adoption and has been the object of intense research work. The literature produced over the years on GE crop safety is large (31 848 records up to 2006; Vain, 2007) and it started to accumulate even before the introduction of the first GE crop in 1996. The dilution of research reports with a large number of commentary papers, their publication in journals with low impact factor and their multidisciplinary nature have been regarded as negative factors affecting the visibility of GE crop safety research (Vain, 2007). The EU recognized that the GE crop safety literature is still often ignored in the public debate even if a specific peer-reviewed journal (http://journals.cambridge.org/action/displayJournal?jid=ebs) and a publicly accessible database (http://bibliosafety.icgeb.org/) were created with the aim of improving visibility (European Commission, 2010).

We built a classified and manageable list of scientific papers on GE crop safety and analyzed the distribution and composition of the literature published from 2002 to October 2012. The online databases PubMed and ISI Web of Science were interrogated to retrieve the pertinent scientific records (Table S1 – Supplementary material). We selected original research papers, reviews, relevant opinions and reports addressing all the major issues that emerged in the debate on GE crops. The 1783 scientific records collected are provided in .xls and .ris file formats accessible through the common worksheet programs or reference manager software (Supplementary materials). They were classified under the scheme given in Table 1, according to the major issues emerging from the literature. Beyond a numerical analysis of the literature, we provide a short explanatory summary of each issue.

General literature (GE gen)

Here we group all the reviews and critical comments offering a broad view of the issues concerning the release of the GE crops into the environment and their use as food and feed, including the regulatory frameworks and risk assessment procedures.

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Table 1. Classification of 1783 scientific records on GE crop safety published between 2002 and 2012.

Topic	No. of papers	%*
General literature (GE gen)	166	9.3
Interaction of GE crops with the environment (GE env)	847	47.5
Biodiversity	579	32.5
Gene flow	268	15
Gf – Wild relatives	113	6.3
Gf – Coexistence	96	5.4
Gf – Horizontal gene transfer in soil	59	3.3
Interaction of GE crops with humans and animals (GE food&feed)	770	43.2
Substantial equivalence	46	2.6
Non-targeted approaches to equivalence assessment	107	6
GE food/feed consumption	312	17.5
Traceability	305	17.1

*Percentage of the total number of collected papers.

Figure 1. Main topics of the scientific papers belonging to the GE env group.
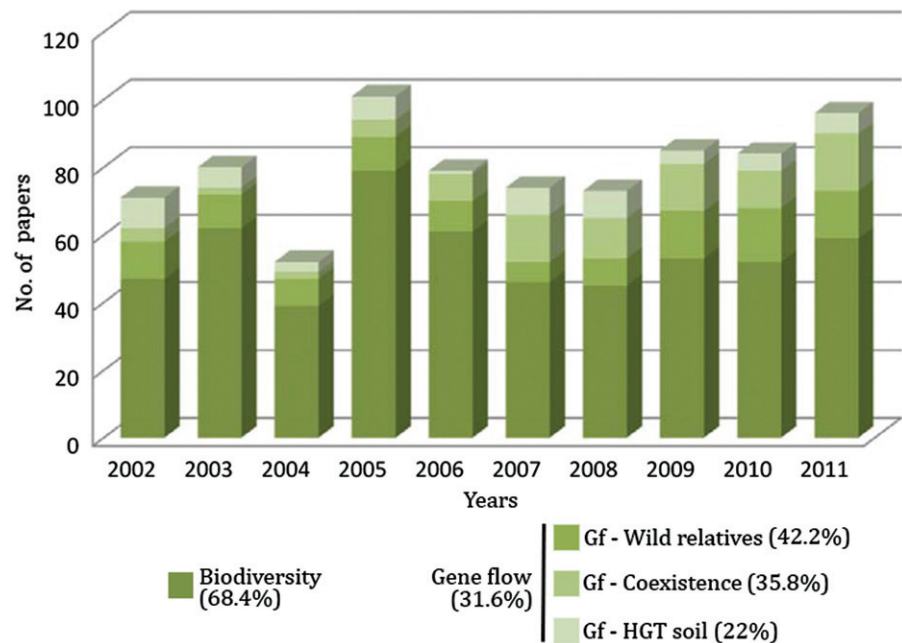
The weight of the **GE gen** section, in terms of number or records, is low in our database (9.3% – 166/1783) compared to **GE env** (47.5% – 847/1783) and **GE food&feed** (43.2% – 770/1783) (Table 1). The literature grouped in **GE gen** reflects the difference between the EU and the US regulatory frameworks: the former is based on the evaluation of the process by which the GE crop is obtained and the application of the precautionary principle, the latter is based on the evaluation of the product. The adoption of such different concepts resulted in the need for new legislation and new authorities in the EU, whereas in the US new regulations were integrated into the existing legislation and institutions (Jaffe, 2004).

Other countries have been inspired by these two systems in developing their own regulatory framework (Ramessar et al., 2008). As a result, the regulations on the release of GE crops into the environment and their use as food and feed are not uniform (Gómez-Galera et al., 2012; Jaffe, 2004; McHughen & Smyth, 2008; Ramessar et al., 2008). This lack of harmonization, and the frequent non-scientific disputes in the media that are not balanced by an effective communication from the scientific and academic world, greatly contribute to enhance the concerns on GE crops.

The EU funded more than 50 research programs in 2001–2010, for a total budget of 200 million euros, with the intent to gain new scientific evidence addressing the public concern on the safety of GE crops. A summary report of these programs highlighted that the use of biotechnology and of GE plants *per se* does not imply higher risks than classical breeding methods or production technologies (European Commission, 2010).

Interaction of GE crops with the environment (GEenv)

Biodiversity

Biodiversity preservation is unanimously considered a priority by the scientific community and society at large. This topic is predominant in **GE env** (68.4%) throughout the decade (Table 1; Figure 1). The literature is highly heterogeneous, since the potential impact of GE crops on biodiversity can be investigated at different levels (crop, farm and landscape) and different organisms or microorganisms (target and non-target) can be considered.

The GE crops commercialized so far are herbicide and/or pest resistant. Glyphosate tolerance obtained by

expressing an *Agrobacterium tumefaciens* enolpyruvyl shikimate 3-phosphate synthase (EPSPS), and the production of insecticidal proteins derived from *Bacillus thuringiensis* (Bt), are by far the most widespread GE traits.

The literature considering the effects on biodiversity of non-target species (birds, snakes, non-target arthropods, soil macro and microfauna) is large and shows little or no evidence of the negative effects of GE crops (Carpenter, 2011 and references therein; Raven, 2010; Romeis et al., 2013). Two reviews about pest resistant GE crops published by Lövei et al. (2005, 2009) reported negative impacts on non-target arthropods; however, these reports have been criticized mainly for the statistical methods and the generalizations between crops expressing Bt proteins (commercialized), proteinase inhibitors (only a transgenic cotton line SGK321 present in the Chinese market) and lectins (not commercialized) (Gatehouse, 2011; Shelton et al., 2009). Negative impacts of Bt plants on non-target arthropods and soil microfauna have not been reported in recent papers (e.g. de Castro et al., 2012; Devos et al., 2012; Lu et al., 2012; Verbruggen et al., 2012 Wolfenbarger et al., 2011). Indeed, the positive impacts have been emphasised.

If we consider the effect of GE crops on the target species, weeds or pests, a reduction of biodiversity is obviously expected and necessary for the success of the crop. For instance, cases of area-wide pest suppression due to the adoption of Bt crops (where also the non-adopters of GE crops received beneficial effects), have been reported (Carpenter, 2011 and references therein). This is also the case of the UK Farm Scale Evaluations (FSE), a series of studies which highlighted that the adoption of a management system based on herbicide tolerant GE crops generally resulted in fewer weeds and weed seeds. These results have been used as proof of the negative environmental impact of herbicide tolerant crops, but indeed they demonstrate the effectiveness of such a management system (Carpenter, 2011 and references therein). On the other hand, higher reductions on biodiversity is generally expected with non-GE crops and herbicide/insecticide applications, because the chemicals used are often more toxic and persistent in the environment (Ammann, 2005).

Concerns have been raised about possible outbreak of resistant populations of target species due to the high selection pressures produced by the repetitive sowing of GE herbicide and pest resistant crops. Glyphosate resistant weeds have been reported (Shaner et al., 2012), as well as Bt resistant pests (Baxter et al., 2011; Gassman et al., 2011). Glyphosate tolerance appears more relevant because, while new Bt proteins are available which can be combined in strategies of stacking, or pyramiding, to reduce the risks of insect resistance (Sanahuja et al., 2011), it seems difficult to find herbicides equivalent to glyphosate in terms of efficacy and environmental profile; therefore, proper management of weed control is necessary (Shaner et al., 2012).

Gene flow

In an agricultural context, gene flow can be defined as the movement of genes, gametes, individuals or groups of individuals from one population to another, and occurs both spatially and temporally (Mallory-Smith & Sanchez

Olguin, 2011). For instance, GE crop plants may be capable of surviving through seed or asexual propagules for years in the field, or they may be able to fertilize sexually compatible non-GE plants (non-GE crop or wild relative plants). The occurrence of gene flow may lead to the spread and persistence of transgenes into the environment or the market.

We have subdivided this topic into three subgroups: gene flow to wild relatives (Gf – Wild relatives), to other crops (Gf – Coexistence) or to microorganisms (Gf – Horizontal gene transfer in the soil). The literature on *Gene flow* makes up 31.6% of the **GEenv** literature and is clearly a ''hot topic'' because its share increased considerably after 2006 (Table 1; Figure 1).

Gf – Wild relatives

This topic represents 42.2% of the *Gene flow* literature (Table1; Figure 1). For estimating the gene flow to wild relatives, the knowledge of several factors is necessary: the reproductive biology of the GE crop, the presence or absence of sexually compatible wild relatives within the reach of GE pollen, and the reproductive biology and the fitness of any hybrid.

The formation of hybrids between GE crops and wild relatives is possible and documented (Londo et al., 2010; Mizuguti et al., 2010). Hybrid fitness determines the chance of transgene introgression, that is, permanent incorporation into the wild receiving population, which was reported in some cases (Reichman et al., 2006; Schoenenberger et al., 2006; Warwick et al., 2008). The risk of introgression should be evaluated case-by-case, considering the features of the transgene(s) incorporated into the GE crop.

The presence of spontaneous populations of GE canola with multiple herbicide resistance genes, probably due to multiple events of hybridization, has been reported (Schafer et al., 2011). Zapiola and Mallory-Smith (2012) recently described a new herbicide tolerant intergeneric hybrid of transgenic creeping bentgrass. Other cases have been reviewed (Chandler & Dunwell, 2008). Pest-resistant GE crops (i.e. Bt crops) may pose more risks than herbicide-resistant crops, because the introgression of a pest resistance transgene may confer fitness advantages to wild plants. Pest resistant wild plant populations may in turn exert selective pressure on the pest populations even in the absence of transgenic crops.

Strategies to mitigate the effect of the transgene(s) in pre- and post -hybridization phases have been proposed (e.g. male sterility, delayed flowering, genes that reduce fitness). However, none of them can be considered completely effective for transgene containment and complete segregation of GE crops is not possible. In any case, there is no evidence of negative effects of transgene introgression so far (Kwit et al., 2011).

It should be kept in mind that the gene flow between cultivated and wild species and its impact on biodiversity is an issue that exists independently of GE crops. The literature is rich in examples of natural invasive hybrids, disappearance of local genotypes (genetic swamping) and resistance to herbicides appearing in wild populations due to natural mutation (Kwit et al., 2011).

Gf – Coexistence

Gene flow from a GE to a non-GE crop can lead to an unwanted presence of the transgene in non-GE products. This issue involves not only the movement of pollen, but also the seeds that could remain in the field and give rise to volunteers, and the mechanical admixture of materials occurring during harvest, transportation and storage. The establishment of populations becoming partially wild (ferals) functioning as a natural reservoir of the transgene must also be considered, as well as the survival chances of the GE crops in the wild.

The coexistence issue goes beyond the matter of gene flow and involves several social and economic aspects, such as the manageability of complex agricultural scenarios where different agricultural systems (organic, conventional and biotech) coexist and a full traceability system is in force.

The collected records on coexistence account for 35.8% of the *Gene flow* literature and their number increased significantly after 2006 (Table 1; Figure 1). Even in the US, the coexistence issue is becoming actively discussed (http://www.gmo-compass.org/eng/news/548.docu.html).

Strategies of coexistence have been investigated for several species, such as maize (Devos et al., 2008; Langhof et al., 2010; Rühl et al., 2011), canola (Colbach, 2008; Gruber et al., 2005), soybean (Gryson et al., 2009), flax (Jhala et al., 2011), wheat (Foetzki et al., 2012), potato, cotton and sugar beet (European Commission, 2006). Maize has been the most intensively studied crop, followed by canola and wheat. Isolation distances, harvesting and post-harvesting practices have been proposed in order to avoid unwanted mixing of GE and non-GE-crop.

The feasibility of a coexistence plan is not only evaluated from a scientific point of view but also considering the extra economic costs due to the containment practices; such extra costs must find compensation in extra income from GE crops (Demont & Devos, 2008). In the EU, the scenario on coexistence is very poor currently, considering that only three GE crops are authorized for cultivation (MON 810 and T25 maize and ''Amflora'' potato), with only MON810 actually commercialized, and Spain accounting for 87% of the entire cultivated surface with GE crops (James, 2011).

Gf – Horizontal gene transfer in soil

Soil microorganisms may uptake the transgene(s) present into the GE crop. In fact, bacteria are naturally capable of acquiring genetic material from other organisms through horizontal gene transfer (HGT). To obtain a GE plant it can be necessary to introduce a gene that makes it possible to select the transgenic cells in tissue culture, by giving them an advantage over the non-transgenic cells. This is frequently achieved with bacterial antibiotic resistance genes that play the role of selectable marker genes (SMGs, recently reviewed by Rosellini, 2012). SMG presence in GE crops is not necessary in the field, and it has raised concerns about the spread of antibiotic resistance genes into the environment and their consumption as food or feed (see below).

The transfer of these genes to bacteria and the possible outbreak of ''super pathogenic bacteria'' resistant to antibiotics has been a matter of detailed investigation by the scientific community. The number of publications on this topic accounts for 22% of the *Gene flow* literature, with a stable presence in recent years (Table 1; Figure 1).

The results obtained so far clearly indicate that soil bacteria can uptake exogenous DNA at very low frequency (10^{-4} to 10^{-8}) in laboratory experiments (Ceccherini et al., 2003; de Vries et al., 2003), whereas experiments in the field did not show any evidence of HGT (Badosa et al., 2004; Demanèche et al., 2008, 2011; Ma et al., 2011). Moreover, in the unlikely event that soil bacteria acquired the resistance to an antibiotic among those currently used in the laboratory to select GE plants, this would not affect the population of natural antibiotic resistant bacteria already present in the soil (D'Costa, 2006; Forsberg et al., 2012) or imply any additional risk for human and animal health.

The substitution of antibiotic SMGs with plant-derived genes (Rosellini, 2011, 2012), their elimination (Ferradini et al., 2011 and references therein) and in general the elimination of any unwanted DNA sequence in the final GE crop is recommended (EFSA, 2011), as proposed with new approaches to plant genetic engineering such as the so-called intragenic (Nielsen, 2003; Rommens, 2004) or cisgenic (Jacobsen & Schouten, 2007) techniques.

Interaction of GE crops with humans and animals (GE food&feed)
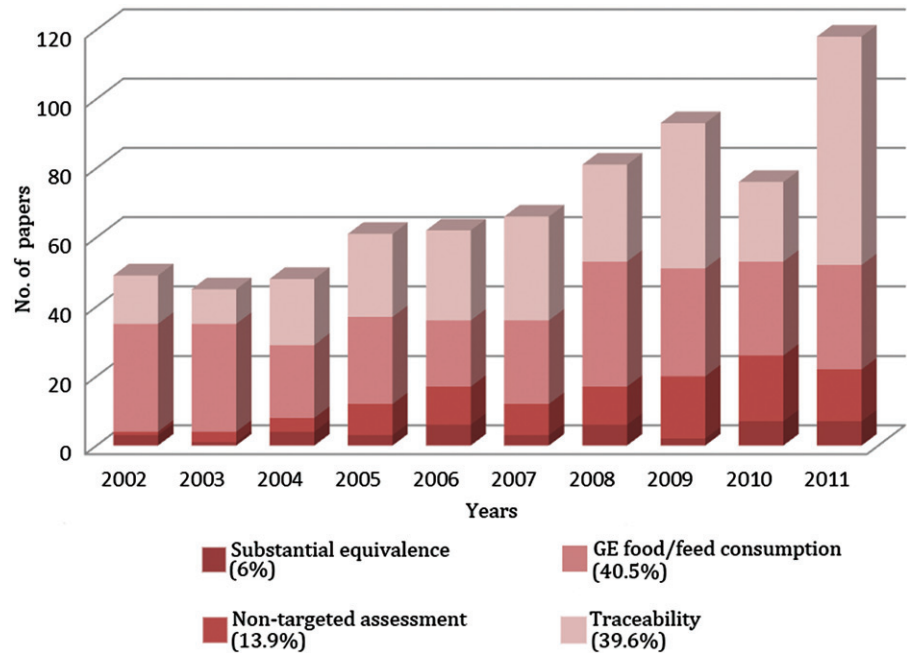
Substantial equivalence

One of the crucial aspects of the risk assessment procedure for a GE crop is to verify if the insertion and/or the expression of the transgene produces alterations in the host organism. The concept of substantial equivalence implies that the GE crop be compared with an isogenic counterpart, that is, the same genotype without the transgene(s).

The demonstration of substantial equivalence is a two-step procedure. First, the GE crop is assessed for agronomic, morphological and chemical characteristics, such as macro- and micro-nutrients, anti-nutrients and toxic molecules. The results of this analysis will provide information on the necessity for further analysis of the nutritive value. Any difference which falls within the range of the normal variability for the crop is considered safe (Colquhoun et al., 2006; EFSA, 2011). This methodology has been agreed internationally (Codex, FAO, OECD, WHO) and involves the quantification of selected molecules, in a so-called ''targeted approach'' (Kok & Kuiper, 2003). If compositional differences are detected, then they have to be assessed with respect to their safety (Ramessar et al., 2007; EFSA, 2011).

The principle of substantial equivalence has been used for risk assessment of the GE crops commercialized so far (Kier & Petrick, 2008; König et al., 2004) and the results support the fact that these crops are equivalent to their non-transgenic counterparts (Parrot et al., 2010).

Concerns have been expressed about the efficacy of the method for detecting unintended effects. Field comparisons in multiple locations have been recommended in order to minimize the differences due to the environmental effects and large data collections have been created (www.cropcomposition.org).

Figure 2. Main topics of the scientific papers belonging to the GE food&feed group.

It is noteworthy that substantial equivalence represents an important common ground of the process-based and product-based regulatory frameworks. This clearly indicates a large consensus amongst scientists worldwide on GE crop evaluation (Kok et al., 2008). Substantial equivalence accounts for 6% of the scientific records collected in **GE food&feed** (Table 1; Figure 2). The literature is composed mainly by the publications produced by the companies that developed the GM cultivars, as part of the authorization process for commercialization. Public availability of the data on which these studies are based should be guaranteed.

Nontargeted approaches to equivalence assessment

The targeted approach to substantial equivalence assessment has an obvious limitation in the number of compounds that are analyzed. On the contrary, the so-called ''–omic'' approaches (transcriptomics, proteomics, metabolomics) can analyze a larger number of molecules (Kier & Petrick, 2008). Several GE crops were compared to their isogenic counterparts using –omic approaches and in some cases differences were observed. However, the interpretation of these results is difficult due to the non-homogeneity of the experimental designs. Moreover, the differences emerging from the –omic analyses have to be cleaned up from the environmental effects and their biological relevance weighted in terms of food and feed safety (Ricroch et al., 2011 and references therein).

It appears that the application of the –omics methods as standard procedure in the risk assessment of GE crop does not actually provide manageable information, and needs further development and validation. In this scenario, the substantial equivalence concept remains a robust and safe reference to determine the presence of unintended effects (European Commission, 2010). The weight of the nontargeted assessment topic increased significantly over the years, especially in 2009–2011 leading to a significant number of publications (13.9%) (Table 1; Figure 2).

GE food/feed consumption

The scientific records grouped under this topic are numerous and constitute 40.5% of the **GE food&feed** literature, clearly indicating the importance of the human health issues. The distribution over the year is uniform, but a peak was observed in 2008, probably due to the scientific fervors that followed the publication of experimental studies conducted by the private companies after 2006 (Table 1; Figure 2). According to the literature, the concerns about GE food/feed consumption that emerge from the scientific and social debates can be summarized as follows: safety of the inserted transgenic DNA and the transcribed RNA, safety of the protein(s) encoded by the transgene(s) and safety of the intended and unintended change of crop composition (Dona & Arvanitoyannis, 2009; Parrot et al., 2010).

Safety of the inserted transgenic DNA and the transcribed RNA

DNA. It is estimated that, with a normal diet, humans consume between 0.1 and 1 g of DNA/day from different sources (e.g. meat, vegetables) (Parrot et al., 2010). This DNA is partly digested, but it can also stimulate the immune-system or promote bacterial biofilm formation (Rizzi et al., 2012). The DNA sequences that drive the expression of the transgenes in the plant cell are generally derived from viruses or bacteria. Concerns have been expressed on the possibility that the transgenic DNA may resist the digestion process, leading to HGT to bacteria living in the gastrointestinal (GI) tract, or translocation and accumulation into the human body and food products from livestock animals. Some considerations can help to put this issue in context:

(a) transgenic DNA is enormously diluted by the total amount of ingested DNA (from 0.00006% to 0.00009%) and is digested like any other DNA (Parrot et al., 2010). In addition, food processing (e.g. baking, frying, boiling)

usually results in DNA degradation (Gryson, 2010; Rizzi et al., 2012) further reducing the amount of intact DNA;

(b) HGT of transgenic DNA to GI bacteria of human and animals is estimated to be an extremely rare event, as confirmed by all the experiments conducted so far (Rizzi et al., 2012). In the unlikely case that this event occurs, the worst scenario is characterized by the HGT of antibiotic resistance genes to GI bacteria, making them resistant to clinical therapies. However, the antibiotic resistance genes found into GE crops today do not present any significant risk to human or animal health (Ramessar et al., 2007), and they are already naturally present into the environment and/or the human/animal GI (EFSA, 2011; Wilcks & Jacobsen, 2010).

(c) DNA fragments can be transferred across the GI barrier. This natural phenomenon has been demonstrated only for high-copy-number genes that have been detected in internal organs, tissues and blood of different animals and even in cow milk (Parrot et al., 2010; Rizzi et al., 2012; van de Eede et al., 2004 and references therein). In humans, the transfer through the GI tract of a high-copy-number gene from rabbit meat has been reported (Forsman et al., 2003).

(d) Transgenic DNA transfer through the GI tract has been reported in the literature in pig, lamb and rainbow trout (Chainark et al., 2006, 2008; Mazza et al., 2005; Sharma et al., 2006;), but in micro quantities and in the case of pigs and lambs with questionable reproducibility due to possible cross contamination (Walsh et al., 2011).

(e) In most studies conducted so far, no fragments of transgenic DNA were detected in any animal-derived products (ILSI, 2008). Only in one case, the presence of transgenic DNA in both ''organic'' and ''conventional'' cattle milk has been reported (Agodi et al., 2006).

(f) No evidence has been obtained to date that DNA absorbed through the GI tract can be integrated into the cells of the host organism and lead to a germ line transfer.

It can be concluded that transgenic DNA does not differ intrinsically or physically from any other DNA already present in foods and that the ingestion of transgenic DNA does not imply higher risks than ingestion of any other type of DNA (European Commission, 2010).

RNA. Along with the DNA also the corresponding transcribed RNAs are ingested and in general the content of DNA and RNA in foods are roughly comparable (Parrot et al., 2010). In the light of recent scientific evidence (Zhang et al., 2012a discussed below) concerns have been expressed about the potential effects that certain types of RNA (small double-strand RNAs, dsRNAs) introduced in some GE crops (e.g. virus resistant, altered oil composition) could have on human/animal health.

The function of such dsRNAs is not to be translated into proteins but to mediate gene regulation through a mechanism termed RNA interference (RNAi). The general mechanism of RNAi is conserved across eukaryotes and is triggered by different types of dsRNAs including small interfering RNA (siRNAs) and microRNAs (miRNAs) (Melnyk et al., 2011).

Recently, Zhang et al., (2012a) reported the first evidence of transfer, through the mouse GI tract, of a food-derived exogenous miRNA (MIR168a) naturally abundant in rice and previously detected also in human blood. This study highlights the unexpected resistance of the rice MIR168a to heat treatment during cooking and to digestion during the transit through the GI tract in the mouse. Moreover, the authors showed significant activity of the MIR168a on the RNAi-mediated regulation of a protein involved in the removal of low-density lipoprotein (LDL) in liver cells (Zhang et al., 2012a). This evidence is still the object of debate at the scientific level and a summary of the major issues are reported here:

(a) miRNAs are naturally present in both animal and plant derived foods/feeds and with a reported similarity to human genes (Ivashuta et al., 2009; Petrick et al., 2013);

(b) Petrick et al. (2013) pointed out that previous studies on feeding rats with rice (Zhou et al., 2011, 2012) failed to provide evidence on any alteration on LDL. However, such studies may be difficult to compare as they were conducted on another species of rodent and with different methodological approaches (e.g. different fasting of the animals and composition of the diet);

(c) although the systemic transmission of dsRNAs has been demonstrated in plants, worms and insects, such transport in mammals is still largely unknown (Melnyk et al., 2011). In humans, the presence of endogenous miRNAs has been documented in microvesicles circulating in the bloodstream and their role in intercellular communication is currently under investigation (Mittelbrunn & Sánchez-Madrid, 2012 and references therein);

(d) the results presented by Zhang et al. (2012a) are not in agreement with that documented in numerous clinical trials involving oral delivery of small RNA molecules. The stability of the dsRNAs in the GI tract and an efficient absorption through the mucosa in order to reach the active concentration of the molecule in the bloodstream, are still the limiting factors in this therapeutic approach (Petrick et al., 2013 and references therein);

(e) some miRNAs are active even at low concentrations and plant miRNAs seem to differ structurally from mammalian miRNAs (Yu et al., 2005; Zhang et al. 2012a; http://www.the-scientist.com/?articles.view/articleNo/31975/title/Plant-RNA-Paper-Questioned/);

(f) interestingly, Zhang et al. (2012b) detected the MIR168a sequence as predominant or sole plant miRNA in public animal small RNA datasets including insects. The authors point out that this may be an artifact due to the sequencing methodology employed (i.e. cross-contamination of the multiplexed libraries).

It can be concluded, that the RNA in general has the same ''history of safe use'' as DNA, since it is a normal component of the diet (Parrot et al., 2010). However, further investigations are necessary to clarify whether the evidence about the MIR168a is due to its unique properties or such conclusions can also be extended to other dsRNAs molecules contained in food/feed.

Safety of the proteins encoded by the transgenes

The expression of the introduced gene(s) leads to biosynthesis of one or more proteins. The ingestion of transgenic proteins has posed some questions about their possible toxic or allergenic effects in humans and animals. The safety of each transgenic protein is evaluated by means of the following analyses:

– bioinformatic analysis to assess the similarity with known allergens, toxic proteins and bioactive peptides;
– functional stability to pH and temperature;
– *in vitro* digestibility using simulate mammalian gastric fluid and simulated mammalian intestinal fluid, following the principle that a digested protein is less likely to be allergenic and absorbed in a biologically active form;
– protein expression level and dietary uptake, to estimate exposure of humans or animals to the protein;
– single dose (acute) toxicity testing and repeated dose (sub-chronic) toxicity testing in rodents using the purified transgenic protein, to predict *in vivo* possible toxic outcome in humans (Delaney et al., 2008; EFSA, 2008).

The results of these analyses are usually part of the documentation that GE crops developers submit to the competent authorities during the approval phase (risk assessment) that precede the commercialization of a GE crop. These data are not always made accessible by the companies or the competent authorities or published on peer-reviewed journals (Jaffe, 2004). However, as indicated by the significant increment of the publications after 2006, it seems that the GE crop developers acknowledged the necessity of an improved transparency (Domingo & Bordonaba, 2011). The experimental data collected so far on authorized GE crops can be summarized as follows:

(a) there is no scientific evidence of toxic or allergenic effects;
(b) some concern has been raised against GE corn MON 810, MON863 and NK603 (de Vendômois et al., 2009; Séralini et al., 2007, 2012), but these experimental results have been deemed of no significance (EFSA 2007, 2012; Houllier, 2012; Parrot & Chassy, 2009);
(c) only two cases are known about the potential allergenicity of transgenic proteins, the verified case of the brazil-nut storage protein in soybean, which has not been marketed (Nordlee et al., 1996) and the not verified case of maize Starlink (Siruguri et al., 2004);
(d) during the digestion process the proteins generally undergo degradation that leads to the loss of activity (Delaney et al., 2008);
(e) even though there are examples of some ingested proteins that are absorbed in minute quantities in an essentially intact form (e.g. ovalbumin, ovomucoid, β-lactoglobulin) (Kier & Petrick, 2008) or proteins that are hydrolyzed into smaller absorbed bioactive peptides (Udenigwe & Aluko, 2012), the consumption of transgenic proteins contained in the authorized GE crop does not result in any detectable systemic uptake (Kier & Petrick, 2008) and transgenic proteins are usually rapidly degraded and not detectable in animal derived products (e.g. milk, meat, eggs) (Ramessar et al., 2007);

(f) pre-screening of transgenic proteins through bioinformatic analyses contributes to avoid the introduction of potentially toxic, allergenic or bioactive proteins into food and feed crops (Delaney et al., 2008; Gibson, 2006; Ladics et al., 2011);
(g) the application of the concept of ''history of safe use'' to the choice the transgene donor organisms may increase intrinsic safety and simplify safety assessment procedures.

Safety of the intended and unintended changes of crop composition

Safety of the introduced change in the GE crop is usually evaluated during the determination of compositional equivalence (Section ''Substantial equivalence''). However, on a case-by-case basis, additional analyses can be requested, such as that of processed foods or feeds, nutritional equivalence and 90-day rodent feeding tests with whole GE food or feed (EFSA, 2008, 2011).

A useful distinction can be introduced here between GE crops modified for input traits (e.g. herbicide or insect resistance) and GE crops with enhanced nutritional characteristics (e.g. increased vitamin content). For the former, the experience suggests that, once the compositional equivalence has been verified, little can be added by the other types of analysis, and nutritional equivalence can be assumed (EFSA, 2011).

On the contrary, for GE crops with improved nutritional characteristics, the nutritional equivalence cannot be assumed, and a nutritional animal feeding test using rapidly growing animals (e.g. broilers) should be conducted to demonstrate the intended nutritional effect. The high sensitivity of rapidly growing animals to toxic compounds may also help to detect unintended effects. The 90-day rodent feeding test is generally performed when the composition is modified substantially or if there are indications of potential unintended effects.

Only GE crops modified for agronomic traits have been authorized for commercialization so far, with the only exception of the ''Amflora'' potato (event EH92-527-1), intended for industrial purpose but authorized also for feed and nonintended consumption (http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=39).

It is noteworthy that, at the moment, the route to the authorization of GE crops intended only for industrial purposes is not fully clarified by the legislation. However, the results of animal tests are routinely presented to the European safety assessment authorities, even if not explicitly required (http://www.gmo-compass.org/eng/safety/human_health/41.evaluation_safety_gm_food_major_undertaking.html).

Recently, Podevin & Jardin (2012) pointed out that the viral promoter P35S, isolated from the cauliflower mosaic virus (CaMV) and used in several GE crops to achieve strong and constitutive expression of the transgene/s, partially overlaps with the CaMV viral gene VI. In some long variants of the P35S promoter this could potentially lead to the production of a residual viral protein. The use of the short version of the promoter is therefore recommended, even if the

bioinformatics analysis of the viral protein has not revealed any relevant similarity with known allergens (Podevin & Jardin, 2012).

An issue emerged about whether the combination of more GE traits in a single crop (GE stacks) may introduce changes that require additional safety assessment. Once safety of the single traits has been established independently, their combination should be evaluated in terms of stability, expression and possible interactions (EFSA, 2011). Weber et al. (2012) pointed out that GE stacks do not impose any additional risks in terms of transgene stability and expression, whereas attention should be focused only on the possible interactions between different traits.

Traceability

This is clearly a ''hot topic'' in **GE food&feed** (39.6%) (Table 1), with the publication rate after 2005 being high and constant (Figure 2). Traceability is defined in the EU General Food Law Regulation 178/2002/EC, inspired to the ISO standard, as the ''ability to trace and follow food, feed, food producing animals and other substances intended to, or expected to, be incorporated into food or feed, through all stages of production, processing and distribution''.

Traceability is a concept already widely applied to non-GE food/feed and it is not connected with their safety (Davison & Bertheau, 2007). It may include mandatory or voluntary labeling for the foods or feeds that contain or consist of GE crops or derived products. Labeling implies the definition of a threshold value, above which the food/feed is labeled according to the regulations in force.

The EU developed the most stringent regulatory framework for traceability of GE crops food/feed and derived products in the world. They have adopted mandatory labeling for unintentional presence of GE material in food or feed, with the lowest threshold value (0.9% based on the number of haploid genomes) compared to other countries (Davison & Bertheau, 2007; Ramessar et al., 2008). Labeling requires the detection and quantification of the GE food/feed or derived product in the tested food/feed or seeds or any other product when applicable. The scientific literature compiled about traceability largely deals with the following issues:

(a) sampling procedures – there are no universally acknowledged sampling procedures (Davison & Bertheau, 2007); this has been the object of a EU funded research programme (Paoletti et al., 2006);

(b) detection method – a large consensus has been established on qPCR (real-time quantitative PCR) -based methodologies that allows detection and quantification at the same time. Other experimental strategies and analytical methods have been proposed (e.g. microarray, Luminex XMAP), but they need further evaluation (Querci et al., 2010);

(c) definition of reference systems – the measurement unit of the GE product concentration depends on the unit used for the certified reference material (CRM) chosen for the analysis. At the moment, in the EU, mass fraction percentages are used for the CRMs, whereas a later recommendation from the EU suggested to use the ''copy

number of transgenic DNA in relation to haploid genomes'', the unit of the legal threshold, so the development of suitable CRMs is necessary (Trapmann et al., 2009);

(d) detection of transgenes in mixtures composed by different ingredients, stacked transgenes and unauthorized events: all these issues require specific approaches and strategies have been proposed. The detection of the unauthorized events is very complex, because it could involve an already known transgene that did not receive authorization or a totally unknown GE event. Unfortunately, asynchronous authorization of GE crops or derived products in different countries does not improve this scenario: a higher degree of international harmonization would be beneficial (Holst-Jensen et al., 2012).

Conclusions

The technology to produce GE plants is celebrating its 30th anniversary. It has brought about a dramatic increase in scientific production over the years leading to high impact findings either in basic research (such as RNAi-mediated gene silencing) and applied research (GE crops), but the adoption of GE plants in the agricultural system has raised issues about environmental and food/feed safety.

We have reviewed the scientific literature on GE crop safety for the last 10 years that catches the scientific consensus matured since GE plants became widely cultivated worldwide, and we can conclude that the scientific research conducted so far has not detected any significant hazard directly connected with the use of GM crops. The analysis of the record list shows that the Biodiversity topic dominated, followed by Traceability and GE food/feed consumption, which contributed equally in terms of the number of records (Table 1; Figure 3).

It is noteworthy that the number of papers on Traceability has increased over the years, overcoming those on Biodiversity in 2011, clearly indicating an increasing demand for methods and protocols for transgene detection (Figure 3). The Gene flow issue also received increasing attention by the scientific community, as a response to the demands of the consumers connected with the coexistence of different productive systems (Figure 3).

It appears that knowledge on Gene flow and GE food/feed consumption would have benefited from a higher number of publications considering their high impact on both environmental and food/feed risk assessment. The difficulties of experimental design and, in the case of Gene flow, the public opposition to field trials, may have discouraged researchers, at least in the EU.

The literature about Biodiversity and the GE food/feed consumption has sometimes resulted in animated debate regarding the suitability of the experimental designs, the choice of the statistical methods or the public accessibility of data. Such debate, even if positive and part of the natural process of review by the scientific community, has frequently been distorted by the media and often used politically and inappropriately in anti-GE crops campaigns. In this regard, Houllier (2012) pointed out that, when
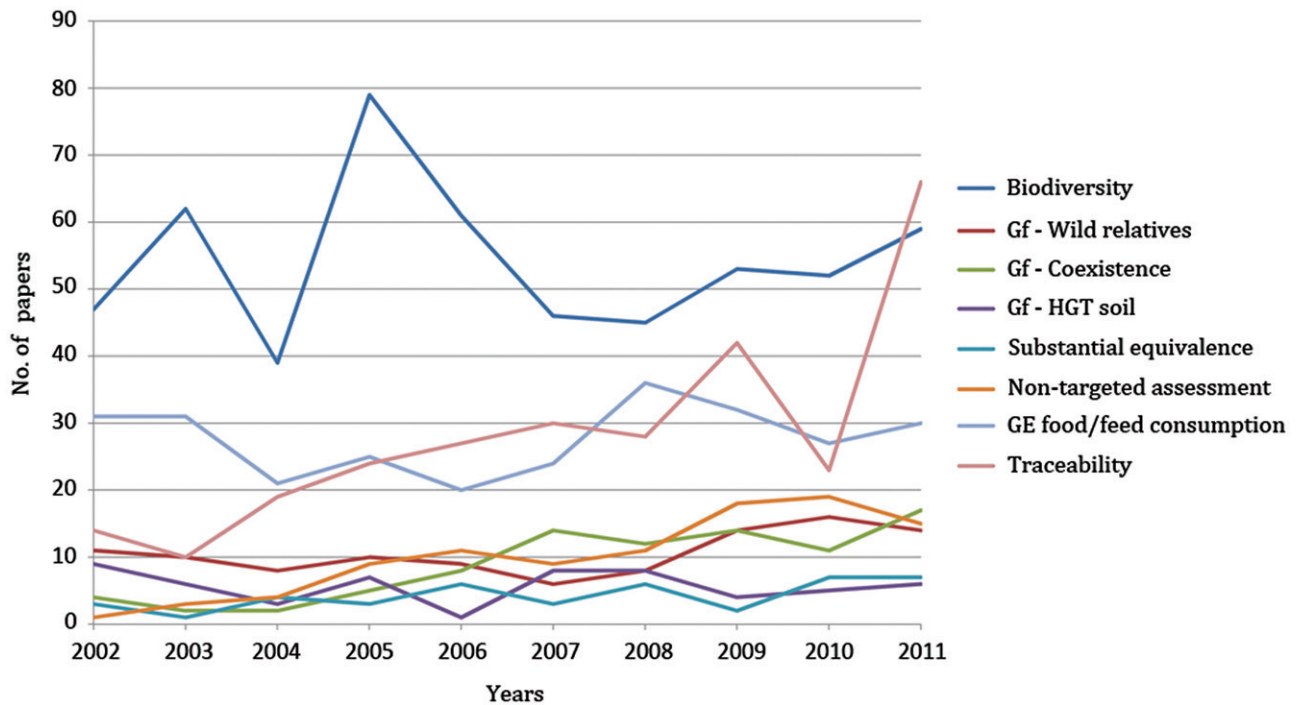
Figure 3. Distribution of the collected scientific papers. Records classified under the *General literature* are not shown.

dealing with ''hot issues'', researchers should take special care in following rigorous scientific standards, avoiding the publication of data not sufficiently peer reviewed by the scientific community.

It is interesting to note that the recent increase of scientific publications about Traceability and Non-targeted assessment (Figure 3) indicates considerable attention to the detection systems and the search for new safety evidence about a relatively low number of new approved GE crops. This likely reflects the consolidation of a situation in which the EU plays mainly the role of the importer of GE crop products from other countries, and enforces a stringent regulatory system.

In the EU, the regulatory burdens for GE crop approval are extremely heavy (Kalaitzandonakes et al., 2007), *de facto* excluding the public sector and minor crops from the development of GE technology. As a result, the number of experimental releases of GE crops is rapidly decreasing (Löchte, 2012) and even large companies are abandoning GE (Dixelius et al., 2012; Laursen, 2012). This scenario is the result of the interaction of complex sociological and psychological factors, risk/benefit ratios, political aspects and an unbalanced scientific communication.

All these factors have to be considered globally and taken into account in a constructive debate on whether the GE crops represent a strategic resource for the future. An improvement in the efficacy of the scientific communication to stakeholders, as clearly demonstrated in the case of the recent case of GE wheat field trials in the UK (Löchte, 2012), could have a significant impact on the future of agricultural GE.

We believe that genetic engineering and GE crops should be considered important options in the efforts toward sustainable agricultural production. Our collection of scientific records is available to researchers, communicators and teachers at all levels to help create an informed and balanced public perception on the hot issue of GE use in agriculture.

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Supplementary material available online

Supplementary Table S1