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Environmental impacts of genetically modified plants: A review

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ABSTRACT

Powerful scientific techniques have caused dramatic expansion of genetically modified crops leading to altered agricultural practices posing direct and indirect environmental implications. Despite the enhanced yield potential, risks and biosafety concerns associated with such GM crops are the fundamental issues to be addressed. An increasing interest can be noted among the researchers and policy makers in exploring unintended effects of transgenes associated with gene flow, flow of naked DNA, weediness and chemical toxicity. The current state of knowledge reveals that GM crops impart damaging impacts on the environment such as modification in crop pervasiveness or invasiveness, the emergence of herbicide and insecticide tolerance, transgene stacking and disturbed biodiversity, but these impacts require a more in-depth view and critical research so as to unveil further facts. Most of the reviewed scientific resources provide similar conclusions and currently there is an insufficient amount of data available and up until today, the consumption of GM plant products are safe for consumption to a greater extent with few exceptions. This paper updates the undesirable impacts of GM crops and their products on target and non-target species and attempts to shed light on the emerging challenges and threats associated with it. Underpinning research also realizes the influence of GM crops on a disturbance in biodiversity, development of resistance and evolution slightly resembles with the effects of non-GM cultivation. Future prospects are also discussed.

1. Introduction

Recent claims of consensus over the safety of genetically modified organisms (GMOs) seems to be an artificial and misguided perpetuated construct (Hilbeck et al., 2015; Domingo, 2016) regardless of contradictory evidences published during last three decades which lead scientific community to reconsider that the debate on this topic isn't 'over' yet. Debates about the commercial introduction of genetically modified (GM) crops started soon after the development of the first transgenic organism (1970s) which led to the development of guidelines for use of recombinant DNA by the US (United States) National Institute of Health (NIH, 2013). Such debates gave birth to some interesting questions needed to be addressed before the release of each and every transgenic organism. Could GM crops outcross to produce weediness? Could they harm wildlife and non-target insects? Could they help to benefit the environment by providing raw materials? Is

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Abbreviations: ALS, Acetolactate Synthase; bp, Base pairs; Bt, *Bacillus thuringiensis*; CaMV, Cauliflower Mosaic virus; DNA, Deoxyribonucleic Acid; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; ERA, Environmental Risk Assessment; FSA, Farm Scale Evaluations; GD, Genetic Diversity; GM, Genetically Modified; GMO, Genetically Modified Organism; HGT, Horizontal Gene Transfer; HR, Herbicide Resistant; ICSU, International Council for Science; IMI, Imidazolinone; Mn, Manganese; nDNA, Naked DNA; NIH, National Institute of Health; NOS, Nopaline Synthase; PAT, Phosphinothricin Acetyltransferase; RNA, Ribonucleic Acid; UK, United Kingdom; US, United States; WYMV, Wheat Yellow Mosaic Virus.

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their environmental impact acceptable or unacceptable? Such arising questions encouraged evolutionary biologists, ecologists, epidemiologists and environmental biologists to broaden the debate for a wider prospective. After the publication of the first report on environmental risks of GMOs (Sharples, 1982), the scientific community started to focus on the impacts which are unacceptable and the tools for assessing such impacts. By reviewing various models of GMO risk assessment, Regal (1986) flawed all concerns which purported that there were no environmental aftermaths produced by GM crops. He claimed that nature has not tried yet all possible genetic variants and that possible risks exist which should be assessed and accounted for. Consequently, for the past three decades, environmental safety has been the subject of research and the assessment of the impact of GM crops on the environment has emerged as an essential component of GMO development and also in the international regulatory process. So, a timely consideration of a present state of knowledge is required as in many parts of the world GM crops have been commercialized and many are in the regulatory pipeline. Generally, risks to the environment could be summarized as (1) risks associated with biodiversity including ecosystem functions effects on soil, and non-target species; (2) risks associated with gene flow and genetic recombination; and (3) risks associated with their evolution i.e. development of resistance either in insect pests or in weeds and *Bacillus thuringiensis* (Bt) crops. The objective of this review is to highlight and discuss the environmental impacts of GM plants. Globally, the scientific community is in intense discussions on the topic and extensive literature of the topic compelled us to illustrate the nature of impacts in detail. We focused to explain primary questions related to direct and indirect effects of GMOs on the environment.

2. Environmental implications of GM plants

The debate for environmental implications of GM crops has been centered on questions such as: what are the potential environmental risks implicated by GM crops? And, if we commercialize GM crops how far it will impart undesirable effects on non-target species? Firstly, toxicity produced by chemicals used with GM crops, is a big challenge to the environment as well as to the inherited plants (De Schrijver et al., 2015). Secondly, such crops can be toxic to non-target species especially to the “friendly” species such as beetles, bees, and butterflies (Yu et al., 2011). Generally, the effect of subsistence, organic or intensive agriculture on the environment is obvious, which strongly demonstrates that GM crops must have implications on the environment. Among many environmental protection platforms, the International Council for Science (ICSU), the GM Science Review Panel and the Nuffield Council on Bioethics (www.nuffieldbioethics.org), approve that GM crops have either positive or negative effect on the environment depending on how and where they are used. The role of genetic engineering in more sustainable crop production as well as natural resource conservation, including biodiversity, is plausible. However, its role in accelerating the damaging effects of agriculture cannot be avoided. Issues of baseline environmental impacts are particularly relevant in relation to the release of transgenic commercial crops (Dale et al., 2002; Domingo and Giné Bordonaba, 2011a; Domingo, 2011b). Direct impacts include gene transfer, trait effects to non-target species as well as wild-life, invasiveness, weediness and genetic recombination of free DNA in the environment. On the contrary, indirect impacts include harmful and side effects of chemical control i.e. reduced efficiency of pest, disease and weed control, the effect on water and soil and global decline of biodiversity (Tutelyan, 2013). Addressed below are the most debatable environmental implications.

2.1. Direct impact of transgenes on environment

2.1.1. Gene flow

Gene flow is considered a major evolutionary force which brings changes in gene frequencies along with mutation, genetic drift and selection (Lu and Yang, 2009). Gene flow can affect the environment by creating a reduction of differentiation between populations as well as an increase in diversity between individuals within a population (Mertens, 2008). The structure of genetic diversity (GD) is also one of the consequences of gene flow (Gepts and Papa, 2003). The introduction of non-native GMOs in the ecosystems pose potential long-term risks to the environment and it is quite difficult to predict their consequences. Scientists from various streams around the globe are concerned with the possibility of transfer of the transgene sequences to related wild species or weeds via horizontal gene transfer (HGT) or hybridization. No doubt case-by-case environmental implications of gene flow are variable but some of the effects of gene flow could be generalized on the basis of general findings in relevance to many cases, such as development of superweeds, evolution of new viral pathogens, instability of transgenes in the environment, creation of GD, evolution of pests and pathogens having resistance to new compounds (Beckie et al., 2012; Yu et al., 2011; Egan et al., 2011). Concomitantly, secondary effects of gene flow also need to be addressed including effects on non-target species, biodiversity disturbance, species displacement and extinction, disturbance in soil micro-environment and species of ecological concern (Layton et al., 2015). The possibility of evolution of new species cannot be neglected and could also lead to an infinite number of biotic interactions (Beusmann and Stirn, 2001).

It is an implicit expectation to consider gene flow from GM crops as it has happened for a millennia between sexually compatible species (Keese, 2008). However, this expectation is based on some basic concepts such as distance between compatible plant species, synchronization of flowering time, ecology of the recipient species and off course sexual compatibility (Han et al., 2015; Gressel and Rotteveel, 1999). Certain features of transgenes make them more suitable to be introgressed into wild counterparts such as dominance, no association with deleterious crop alleles, and location on shared genomes and/or on homologous chromosomes (Hartman et al., 2013; Stewart et al., 2003). Mathematical models of pollen movement are being developed to forecast the possibility of gene transfer through this mechanism (Dale et al., 2002; Raybould and Gray, 1999). Examples of such investigations are reported in rapeseed, maize, cotton, wheat, barley, beans and rice (Yan et al., 2015; Han et al., 2015; Lu and Yang, 2009). Pollen-mediated gene transfer solely depends upon pollination biology of the plant, amount of pollen produced, mating system between donor and recipient species, outcrossing rate, relative densities of donor and recipient species, types of vectors, wind, air turbulence, water current, temperature, humidity and light intensity (Papa, 2005; Mercer et al., 2007; Hancock, 2003). From a recent investigation conducted by Dong et al., (2016) they reported that a pollen-mediated gene flow was significantly affected by wind direction. Furthermore, a drastic decrease in pollen-mediated gene flow was reported with increasing distance from the pollen source in WYMV- resistant transgenic wheat N12-1. In transgenic corn, canola and creeping bentgrass, pollen transfer rate decreased rapidly when the distance was increased just by 30 m, 20 m and 20 m respectively (Goggi et al., 2007; Knispel et al., 2008; Van de Water et al., 2007). Highest gene flow frequency has also been reported in creeping bentgrass and rigid ryegrass as a result of pollen flow with the pollen donor just 2000 and 3000 m away (Van de Water et al., 2007; Busi et al., 2008). Comparatively low frequency of gene flow has been witnessed in self-pollinated crops than cross-pollinated crops (Warwick et al., 2009) as in the case of pollen-mediated direct and indirect gene flow from rice to red rice weed and vice versa was < 1%. Another two possible mechanisms responsible for gene flow are seed mediation and vegetative propagule-mediation methods (Lu, 2008). Seed-mediated gene transfer is supported by human error during sowing, harvesting or post-harvest handling or presence of adventitious plants (Schulze et al., 2014). Adventitious presence of herbicide resistance genes in farm harvested seed has been observed in corn, wheat and canola (Petit et al., 2007; Gaines et al., 2007; Friesen et al., 2003).Vegetative propagule-mediated transgene transfer is caused either by vegetative organs of plants or by various animals (Schulze et al., 2014). Scientists argue that whether such a flow of transgene really matters or not and if it indeed matters, what would be the consequence? As being debatable as mentioned above, such events have been going on in nature between conventional crops and land races without constitution of any environmental problem. Introduction of new traits and novel genes into ecosystems as a result of genetic engineering raises additional concerns allowing flow of genes into diverse crops with variable outcrossing potentials (Ellstrand et al. 2003b). Here we discuss the detailed impacts of gene flow on the environment accompanied with relevant underpinning research.

2.1.1.1. Transgene×wild hybridization. Scientists acknowledge the possibility of flow of transgenes because of the plants ability to hybridize with sexually compatible species and release of hybrids in the environment as well as from the spread of transgene contamination. Disturbance in the ecosystem can be attributed to persistence of possible transgenic×wild hybrid having competitive advantage over the wild population. Theoretically, for such a hybrid to be developed under natural conditions, a rare hybridization event would be sufficient (Cruz-Reyes et al., 2015) and the developed hybrid could have higher fitness compared to its parents. Fitness is the relative ability of a hybrid to survive and subsequently reproduce in an environment (Heil and Baldwin, 2002; Haygood et al., 2003). Development of such a hybrid depends on certain factors such as flowering period synchronization, reproductive fitness of the hybrid and its survival rate (Lu and Yang, 2009) (Fig. 1). Fitness could decrease in first hybrid progeny F1 but is recovered in the next hybrid progenies as observed in imidazolinone (IMI) resistant sunflowers (Presotto et al., 2012). An ample amount of fitness differences were observed in *Brassica rapa* × *Brassica napus* F1 hybrids and both parental species (Hooftman et al., 2014). Risk of unintended gene transfer is higher in regions where the crop species were originated and had wild relatives (Lu and Snow, 2005). Detection of NOS (Nopaline Synthase) terminator and CaMV (Cauliflower Mosaic Virus) 35S promoter in Mexican maize land race populations strengthened the idea of gene transfer from GMO to land races and wild relatives (Pineyro-Nelson et al., 2009). After transgene flow to host plant genomes, certain factors such as hybrid vigor, selection and heterosis will play a role in determining transgene frequency in wild populations. Fitness in hybrids will solely depend on competency to cross with wild counterparts or related species, the life cycle of hybrids and their parents, fecundity, changes in seed bank survival rates, seed persistence and seed dormancy (Lu and Snow, 2005; Hooftman et al., 2014). Fitness costs in wild plants and crops must be different due to their diverse genetic backgrounds and possible causes are pleiotropy, physiological costs of the new traits or effects of particular insertion sites within the genome and genetic changes in plant genomes as the consequence of insertional mutagenesis (Schnell et al., 2015). Fitness of crop×wild sunflower hybrids was greater in relative competitive conditions of wheat intercropping as compared to crop lines and it was greatly affected by genotype×environment interactions (Mercer et al., 2007). Influence of the above mentioned random and unintended effects to other related traits is not negligible but might remain unnoticed until the establishment of transgenes as wild populations; one such example is the transgenic sugar beet × swiss chard hybrids for their bolting pattern (Ellstrand, 2003a). A collection of triploid individuals in commercial canola fields in Chile put forth the evidence of GM × wild interspecific hybridization (Prieto, 2006). Gressel (2000) also hypothesized a fitness penalty in *Arabidopsis thaliana* just arising out of target site resistance and increased pollen donating ability to nearby non-GM mother plants. Such cases of gene flow are always accompanied by some kind of selection pressure against herbicides, insecticides, abiotic stress or pathogens. However, even if there is no kind of selection pressure introgression, persistence of transgenes into wild populations is still possible due to the regaining of selective fitness by successive backcrossing (Wang et al., 2001) and was noticed by Schulze et al., (2014) who reported the presence of glufosinate-resistant (particularly, events MS8×RF3, MS and RF3) feral plants of oilseed rape in Switzerland even if there was no transgenic oilseed rape in the surrounding area at the time of sampling. Genetic bridge on the other hand is also responsible for the gene flow as crop hybrids and a sexually compatible wild plant can also deliver transgenes directly to non-hybridizing species (Lu and Snow, 2005; Tutelyan, 2013; Darmency, 2000). Among transgene introgression target families, the *Poaceae* and the *Brassicaceae* families have been reported to have a maximum number of natural hybrids (European Food Safety Authority, 2016). Ellstrand (2002) reported that at least 44 cultivated plants could possibly cross with one or more wild relatives in different agro-ecological zones of the world. Twenty-eight cultivated species including 22 world food crops have been witnessed to have natural hybridization with one or more wild relatives. He further confirmed hybridization with related wild plants for 83 species, due to the presence of sympatry, it was evident that 48 species had something more than just morphological intermediaries. Recently, the outcrossing potential of 11 GM crops with vascular flora in Chile was documented by Sanchez et al., (2016). 810 of 3505 introduced species and 824 of 4993 native species had inter-relationships either based on genus or species correspondence. Based on above reports, the level of risk probability in 25 different crops is presented in Fig. 2.

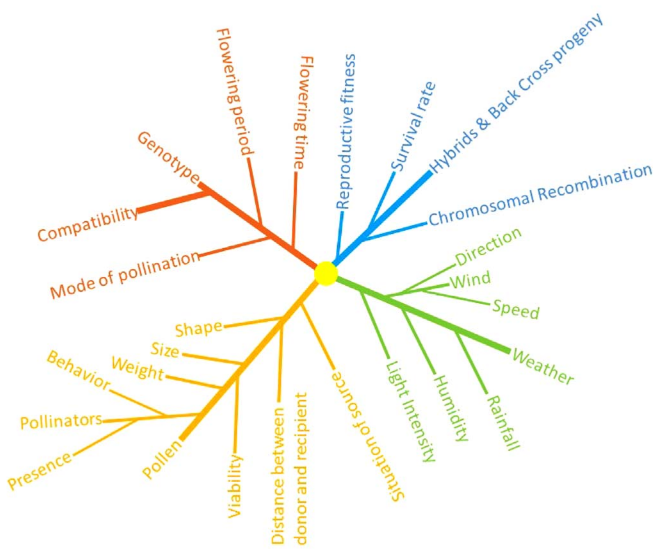

Fig. 1. Factors (weather conditions, pollination conditions, genotype-compatibility, backcrossing) influencing rate of gene flow through hybridization.

Additionally, hybrids progeny of GM × sexually compatible species may carry hemizygous allelic conditions that have a possibility of not expressing at the phenotypic level unless it reaches a homozygous condition as a consequence of additional self or cross-pollination events (Sanchez et al., 2016). GM Science Review Panel (2003) confirmed the absence of such hybrids which could have become invasive in the wild in the UK. Furthermore, no such transgene transfer has been documented in maize, cotton, canola and soybean (Heuberger et al., 2010). However, in the case of *B. napus,* herbicide resistance

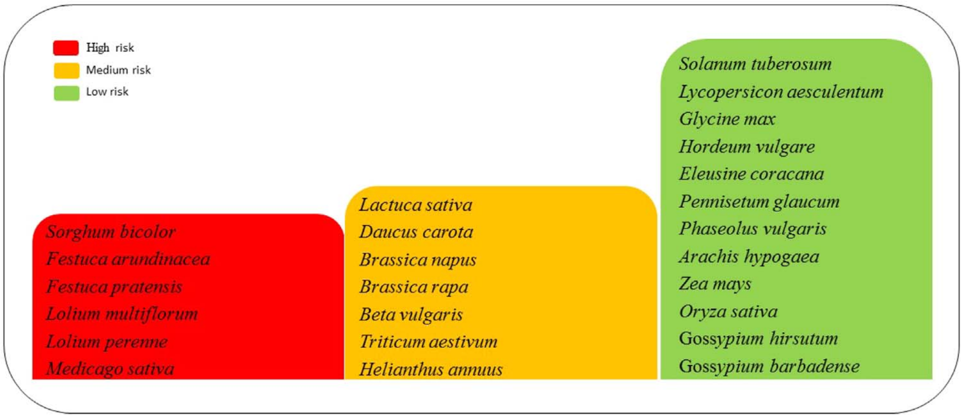

Fig. 2. Level of risk probability of gene flow in 25 common crops based on molecular evidences (Stewart et al., 2003), pollen movement (Raybould and Gray 1999) and biology of the crop (Hancock 2003; Gressel and Rotteveel 1999; Mertens 2008). Based on these reports species were arranged into three groups i.e. high, medium and low risk. Figure was drawn using Microsoft Office® tool PowerPoint.

transgene transferred to its wild weed type relative *B. rapa* in Quebec and from its persistence for the following six years, it was observed that no herbicide selective pressure in natural conditions occurred (Warwick et al., 2008). Based on the above reports it is obvious that hybrids may develop by introgression of GMO with its wild relatives and hence the possibility of transformation of resistant genes exists.

2.1.1.2. Transgene stacking. There is an increase in the usage of GM crops around the globe with the development of transgenic plants with improved resistance to herbicides and insect pests. The area covered by single transgenic trait, such as glufosinate tolerance, is still high but the relative percentage of GM crops with stacked traits (herbicide tolerance, insect resistance, fertility restoration, male sterility, mannose metabolism, a visual marker and antibiotic resistance) has been increasing. Only in 2012, 43.7 million hectares were planted having stacked biotech traits with an average year-to-year increase of 31% (www.isaaa.org). Many commercial companies such as Bayer Crop Science, Syngenta, Pioneer, Monsanto and Dow Agro Sciences are pursuing to achieve GM crops with stacked traits (Supplementary table 1). Ecological and environmental consequences of transgene stacking also need to be accounted for. Transgene contamination may include approved transgenic constructs as well as sequences and constructs that have not been approved in a given country (De Schrijver et al., 2007). Kok et al., (2014) categorized three possible risk scenarios from plants with stacked traits. Those are gene stability, changes in the level of gene expression and synergistic or antagonistic effects. Initially, the likelihood of a stacked gene escape may be low, but in the long term, it is likely that multiple transgenes will be found in wild plant populations (De Schrijver et al., 2007). In rare cases, nuclear-encoded and plastid-encoded genes may even be combined (Halpin, 2005). Accidental stacking, as well as intentional breeding between sexually compatible GM plants, may lead to accumulation of many genes in the same area. Consecutive generations of related and sexually compatible weed species would have a chance to receive transgenes with wide ranges of modes of action such as resistance to pests, different stresses, tolerance to herbicides etc. and would persist in the environment more forcefully (Mertens, 2008). Recent developments in plastid genetic engineering have enabled expression of multiple genes in a single operon. On the contrary, gene escape from such events will pave ways to introgress maybe the whole stack of transgenes that are often related to a single metabolic pathway. Environmental risk as a consequence of such gene flow could develop resistant and tolerant weeds just in a single generation (Bock, 2007). Significant changes in endogenous gene expression and at the protein level can be observed in GM plants with stacked traits as compared to a single event or conventional counterparts. Expression of two stacked genes (Enolpyruvulshikimate-3-phosphate synthase and cry genes) resulted in alterations in the energy/carbohydrate and detoxification pathways in maize. Both stacked genes had 34% lower expression when compared to single event hybrids (Agapito-Tenfen et al., 2014). Some reports suggested that these reduced expressions could lead to resistance development in target insect-pests (De Schrijver et al., 2015). Synergistic and antagonistic effects of stacked transgenes may offer risks at two levels. Firstly, interaction of proteins or stacked event components at a GM plant component level may affect some pathways such as high oleic acid GM soybean may cause synergistic or antagonistic effect on other components of oleic acid pathway Secondly, the effect can be anticipated at the cellular level where expression of transgenes may affect levels of cell components (Kok et al., 2014). However, at the cellular level, the risk cannot only be associated with stacked traits, as single event transgenes could also offer the same risk. Compared to self-pollinated crops, open pollinated crops are at higher risk of developing polygenic transgene traits as a result of recombination of multiple transgenes. What would be the possible impact of such gene stacked gene flow on the environment and biodiversity? Well, the most important thing will be the management of weeds and stacked transgene volunteers. Stacked transgene volunteers have already been persisting in Canada (oilseed rape) resistant to different herbicides (Dietz-Pfeilstetter and Zwerger, 2009). So the question is, how could such an environmental threat be managed? Orson (2002) suggested that it will be inevitable to practice such volunteers in the field of agriculture. De Schrijver et al., (2015) suggested theoretical worst-case scenario tests to estimate the effect of stacked Bt proteins on non-target invertebrate species. He emphasized on the fact that current knowledge of Bt toxin interactions is limited and should be evaluated via more precise data. Schuppener et al., (2012) reported that stacked maize (Cry1A.105 and Cry2Ab2) against lepidopteran and chrysomelidae had no significant effect on tortoiseshell butterfly in European agrarian landscapes. Another study involving Bt11×MIR604 maize; expressing Cry1 Ab and mCry3A proteins, revealed implausible results that cultivation of stacked GM maize had no greater differences than respective single maize events (Raybould et al., 2012). Combined toxicological impact of Cry1F and phosphinothricin acetyltransferase (PAT) proteins from TC1507 maize was considered on dairy cows, beef heifers, swine, laying hens, broiler chickens, and rodents. The report presented negligible or no allergenic or toxic effects to humans or either of the organisms studied. The extent of gene flow and HGT was not detected (Baktavachalam et al., 2015).

2.1.1.3. Horizontal gene transfer. Stable transfer of genes other than a parent to offspring (sexual/asexual) is considered as horizontal gene transfer (HGT) (Keese, 2008). The transfer occurs by the passage of donor genetic material across cellular boundaries, followed by heritable incorporation to the genome of the recipient organism. The most popular genetic transformation strategy i.e. *Agrobacterium tumefaciens* is the result of HGT (Conner et al., 2003). Naturally many diverse mechanisms are involved in the uptake and establishment of genetic material in addition to transduction, transformation, and conjugation. Environmental situations including soil, freshwater, seawater, wastes from industry and animals, plant surfaces, human and animal intestines and saliva and food products may aid HGT (Burmeister, 2015). With the advent of genetic engineering, risks associated with environment and biodiversity are being questioned by considering possibilities of HGT. The role of HGT in microorganism evolution as well as macroorganisms under natural circumstances has already been acknowledged and it is a well-understood fact that mechanistic HGT has no direct adverse impacts but the fitness changes of the recipient organism create drastic effects (Conner et al., 2003). Keese (2008) gave detailed explanations of risks associated with HGT and possible factors which play a role in gene transformation. HGT from GM plants has raised additional concerns about the possibility of transgene transfer to another organism. Such gene flow could become a potential risk to mankind as well as to the micro and macro environment (Conner et al., 2003). Possible cases of HGT could involve the transfer of antibiotic resistance transgene to pathogens and transgene flow to viruses and/or to humans (Ho et al., 2000). Such gene transfers could take place in soil, water as well as a gastrointestinal tract of humans or animals. However, these cases are still highly speculated and detailed experimental evidence are awaited. It is important to consider the interplay of alleles among bacterial communities with special consideration of HGT which highlights the fact that ecological barriers to allele transfer could be surpassed in different ways. Such a strategy is adopted by many bacterial species to maintain population genetic similarity, however, this characteristic phenomenon poses a threat to the environment when considered in the context of GM plants and bacterial interaction at a microclimatic level. Acquirement of multiple antibiotic resistance in a wide range of bacterial populations due to the widespread use of antibiotics in humans as well as animals medicine is another raising concern (Lawrence and Retchless, 2009).

Transgene transfer from GM plant roots and leaves to microorganisms has been demonstrated by Tepfer et al. (2003). Such studies have confirmed that Arabidopsis, oilseed rape, tobacco, alfalfa and carrot could transfer genes (nptII gene system as a marker) to soil bacterium *Acinetobacter* spp. Many experiments revealed that intact leaves of tobacco having plastid transgenes could produce bacterial transformants consistently. Certain factors have been considered important in HGT such as size of the transgene, nuclear or plastid transgene, sequence mosaicism, selective pressure, transgene copy number, genome size of the recipient species, codon usage between donor and recipient species, type of promotor used in the insert, compatibility of RNA and protein synthetic machinery. (Tepfer et al., 2003; Daniell et al., 2001). Natural GM sweet potato (*Ipomoea batatas* [L.] Lam.) harboring many *A. tumefaciens* DNA (particularly two T-DNA regions i.e. *Ib*T-DNA1 and *Ib*T-DNA2) sequences strengthen the hypothesis that HGT can be a possible route of transgene movement from microflora to GM plants and vice versa (Kyndt et al., 2015). These regions were transferred naturally when *A. tumefaciens* infected sweet potato during the course of evolution. Recent investigations targeted at the transfer of CaMV-P35S promoter from a GM diet to blood in liver and brain of male Wistar albino rats suggested that this promoter have affinity of incorporation. The report suggested that larger segments had a higher incorporation frequency than shorter sequences and affinity increased with the increase of feeding duration (Oraby et al., 2015). Many researchers are in a debate that HGT frequency from plants to prokaryotes is as low as 2×10^{-17}, while some scientists argue that 10 recombinants per 250 m^2 could be predicted considering a transgene transmission frequency of 10^{-17} (Mertens, 2008). Matthews et al., (2011) predicted HGT of *Rhodnius prolixus* less than 1.14×10^{-16} per 100,000 generations with 99% certainty level. Apart from traditional marker transgenes, novel transgenes having no natural counterparts i.e. those genes which are being engineered for production of pharmaceuticals, chemicals and vaccines, necessitate investigation in relation to HGT which may frequently include unique combinations of toxin protein domains and regulatory elements, derived from diverse species which will probably differ considerably from those arising by natural evolution. HGT of dsRNA from GM crops to other related organisms should also be accounted for (Heinemann et al., 2013).

2.1.1.4. Structure of genetic diversity. Gene flow can affect the environment by creating a reduction of differentiation between populations as well as an increase of diversity between individuals within a population. The structure of GD or so-called “domestication bottleneck” is also a consequence of gene flow and could be determined by considering the life history and demographic factors of domesticated crops (Lu and Yang, 2009). Those crops which domesticated from a small initial crop population exhibit reduction of genetic variation referred as domestication bottleneck. The natural flow of genes from wild to domesticated crops is the main driving force for partial restoration of GD and introduction of new alleles (Marri et al., 2007). Additionally, such gene flow plays an important role in evolution. Perversely, by the advent of modern genetic engineering and plant breeding, traits including resistance to many pests and pathogens and quantitative trait loci for quality and yield have been incorporated in crop plants which are grown at commercial scale. The flow of such transgenes from GM crop plants to wild relatives cause reduction of GD and sometimes complete genetic extinction of wild populations (Gepts and Papa, 2003). Gene frequencies are affected by certain factors mainly mutation, selection, genetic drift and migration (Papa and Gepts, 2004). Migration of gametes via pollen movement between GM plants to wild relatives could be a strong factor to reduce GD between subpopulations. With such migration of transgenes, gene frequencies across whole genomes of the recipient species will be disturbed by genetic recombination mainly on target loci (Cruz-Reyes et al., 2015). Therefore, the question to be addressed here is what is the scenario related to the uninvited effect of transgene flow on GD? Lu and Yang (2009) mentioned two such possibilities. Firstly, generation of selective sweeps could be the after effect of the strong selection of fitness augmenting transgenes. Such a situation could arise when portions of genomes of host species linked to transgenes are displaced (Papa and Gepts, 2004). Secondly, population decline or local extinction of nearby wild populations is possible because of the enormous influx of fitness-reducing transgenes. The first possibility has more chances in self-pollinating GM plants as in the case of cross-pollinated plants where there are more chances of allele dilution and mixing (Haygood et al., 2003). GD of rhizosphere bacteria can also be affected in the GM cropping system. However, no such detailed report has yet appeared. No significant genetic variations were detected in major rhizospheric bacterial groups such as Proteobacteria, Actinobacteria, Chloroflexi and Firmicutes in the root zone of MON810 maize (Ondreickova et al., 2014). Overall, the possibility of controlling disturbance in GD will be regarded as the farmer’s decision by compensating crop production with non-GM crop plants rather than agreeing with current scenarios of widespread mono-crop GM cultures.

Magnitude and quality of gene flow in any particular case unfolds the possible risks associated to it. From the above discussion, it is now clear that gene flow is a strong evolutionary force and strongly demand to devise special containment strategies to reduce it as much as possible (Ellstrand, 2014). Possible strategies include (1) isolation zones or border areas (2) trap crops (3) molecular strategies such as restricting flower opening, chloroplast engineering, male-sterility, genome incompatibility, seed sterility, apomixes, transgene excision and cleistogamy (Morris, 1994; Staniland et al., 2000; Daniell, 2002; Husken et al., 2010).

2.1.2. Fate of naked DNA

nDNA encoding a resistance or tolerance trait can possibly persist in the natural environment (Barnes and Turner, 2016). There are several potential sources of nDNA to be transferred, such as compost of GM plants and manures of farmed animals having GM fodder/forages (Gulden et al., 2005). There exists another possibility of transgene movement to natural habitats by meat and milk from animals fed GM diets. In addition to nDNA, naked dsRNA from GM plants produced by dsRNA-mediated silencing can pose additional risks (Heinemann et al., 2013). Once nDNA has escaped, its persistence in the environment will solely depend on certain factors i.e. size of transgene, type of DNA (plastid/nuclear), kind of mineral or particle in soil to which DNA will bind, physiological state of recipient micro/macro-organism, stress on recipient microbe as well as availability of nutrients, pH of soil, amount of humic acid and soil temperature (Dale et al., 2002; Mullany, 2000). The size of naked and degraded DNA (possibly transgene and its regulatory sequence) and its facilitating flanking DNA sequences are essential factors for successful integration. To gain a perspective of the environmental impact of nDNA let's consider the amount of such DNA being added in the environment. In contrast to immense quantities of DNA from non-GM plants added in the environment, through pollen, leaves, fruits and compost and decaying plants, the relative amount of DNA driven from GM plants is quite low (Dale et al., 2002). Once nDNA has escaped from a GM host and reached the environment, what could be the possible damage to the environment and what is the risk? Can this DNA create disturbance in ecosystems? Well, the risk from such events is not negligible. Firstly, such naked-extracellular DNA could be a source of the gene pool for surrounding microbial communities, especially bacteria and fungi having the competency of natural intake of DNA. Secondly, the most devastating risk could be the viral pathogens (especially bacteriophages) residing in microflora that could receive nDNA. Thirdly, gene transfer from bacteria residing on GM crops to microbes in intestines of animals feeding on GM crops is another possibility (Dale et al., 2002). Intake of GM DNA in dairy cows fed with transgenic Bt maize was 0.000094% of the total DNA intake which was almost 54 μg/day. While daily intake of non-GM DNA in cows was found to be 54–57 g/day (Phipps et al., 2002). Although the possibility of such a transfer is quite negligible because of nucleases in the animal's intestine would degrade the nDNA (Flachowsky et al., 2005a). Degraded DNA fragments of 680 bp were detected within 28 days in maize cob silage, while only 194 bp fragments were observed in whole plant silage up to 35 days (Einspanier et al., 2004). This fragmented DNA was instantly degraded in the animal digestive tract in response to various acids, endonucleases, and microbial activities. A case study of detecting CP4EPSPS in sheep fed with Round Ready canola resulted in the detection of 527 bp fragments after 2 min (Alexander et al., 2004). So, the risk probability in an animal's digestive tract is quite negligible. However, there is a possibility that these small fragments can be endocytosed by microbes residing in animal intestines and can be incorporated into host microbial genomes. Thirdly, highly degraded DNA segments may introduce amino acid substitutions or indels to recipient bacterial genomes either by transposition or homologous recombination apart from the fact that these highly degraded segments are unlikely to transfer novel protein encoding abilities (Van Hoek et al., 2011). Lastly, if the decomposed GM material is exposed to aquatic ecosystems, then perhaps in the gastrointestinal tract of aquatic animals and fish, fungal species could possibly up take nDNA (Mullany, 2000). Persistence of nDNA from Bt corn (event MON863) containing Bt3Bb1 and nptII genes and DNA from plasmid Pns1 in water was reported to decrease by two orders of magnitude within > 4 days (Zhu, 2006). As far as the persistence in agricultural ecosystems concern, the possibility of nDNA perseverance is not zero. The persistence of nDNA in root zones of Roundup Ready GM corn and soybean is for a very short duration of 26.7 h if temperatures are high (> 15 °C) while, its persistence increases when temperatures are < 15 °C and frequent rainfalls can distribute there DNA into various soil layers and across the agricultural fields (Gulden et al., 2005).

2.1.3. Weediness

Change in invasiveness or persistence of the crop in agricultural, as well as natural habitats, is another rising concern which exerts severe and irreversible effects on biodiversity. Establishment of a transgene or transgene×wild hybrid as a weed in other fields or other habitats is referred as weediness. Weediness is one of the potential effects of adoption of herbicide-resistant (HR) crops (Ammann et al., 2000). ICSU, GM Science Review Panel agreed that domesticated crops are at low risk of being established as a weed because domesticated traits often have less fitness in the wild. However, recent studies support that domesticated crops could escape from cultivation (ferality) and become a potential weed. Traits like fast growth rate, self-compatibility (crop traits) could favor weediness (Ellstrand et al., 2010, 2013; Mercer et al., 2014). A gain of herbicide resistance gene through hybridization with GM plants could lead towards its persistence in the agricultural habitat (Guan et al., 2015). There exists a contradiction among scientists about the establishment of transgene recipient as weeds in the environment. For example Williamson et al. (1990) reported that small genetic modification through GMO × domesticated crop hybrids could cause large ecological alterations. On the other hand, Luby and McNichol (1995) argued that from the addition of a single transgene it is unlikely to establish a crop as a weed. Based on the risk of increased fitness, certain traits are strong candidates which can enhance chances of competitiveness, such as tolerance to herbicides, resistance to various stress, pathogens and pests and traits responsible for enhanced growth (Yang et al., 2012). Considering dispersal, plants having characteristics like perennial, hardy, prolific, and competitive and having the ability to withstand a range of natural habitats could be considered as high impact plants (Mertens, 2008). Furthermore, the rate of weediness through gene flow relies on the frequency of hybridization and net selective effects of target transgenes (Lu and Yang, 2009). Certainly, some plant species exist as weeds as well as crops (Ammann et al., 2000). So, what could be the risk regarding such species? Obviously, a change in the habitat could exert potential pressure for the evolution of a weed from a cultivar or from a closely related feral plant. Plants can develop several herbicide-resistance mechanisms, such as herbicide detoxification, changes in the intracellular compartmentation of herbicides, target site insensitivity, reduced herbicide entry, reduced herbicide translocation and target site overproduction (Guan et al., 2015). According to the GM Science Review Panel, “Detailed field experiments on several GM crops in a range of environments have demonstrated that the transgenic traits do not significantly increase the fitness of the plants in semi-natural habitats”. Disease or pest resistances are the traits which could provide a fitness advantage to weeds and could have negative environmental penalties, but the possibility is little as per present evidence. Current evidence is insufficient for determination of such probability and it needs more experimental investigations and field surveys. In the case of decreased ambient selection pressure of selective insect in *Bt/CpTI* GM rice in the intensive cultivated agricultural zone, the hybrid progeny had limited fitness advantages (Yang et al., 2012). With zero herbicide selection pressure, escaped herbicide resistance transgene from GM soybean to its wild counterpart (i.e. *Glycine soja*) can still persist in nature (Guan et al., 2015). A remarkable case of amaranth (*Amaranthus palmeri*; a cotton weed) which was reported first in 2004 in Georgia, spread to 76 countries within next the 7 years (Gilbert, 2013). This Report also revealed that 24 glyphosate tolerant weeds have been identified after release of many RT crops since 1996. Interestingly, an improvement of

8.9% environment impact quotient was recorded by PG Economics from 1996 to 2011. A chronological increase in resistant weeds at a global scale from 1955 until 2014 has been published by WeedScience.Org (2014). The report describes that around 145 plant species have become resistant to eight herbicide groups including acetolactate synthase (ALS) inhibitors, triazines, Acetyl-CoA Carboxylase Inhibitors, synthetic auxins, bipyridiliums, glycines, ureas, amides, and dinitroanilines. Current GM crops are experiencing the most extensive risk assessment studies so, likelihood of invasiveness of these herbicide tolerant crops in natural or agricultural habitats could be speculated (Dale et al., 2002). Although the risk of pervasiveness or invasiveness is regarded relatively low, there are some possible biological changes that could lead to weediness such as tolerance to extreme regimes of temperature, water and soil salinity, modification in seed propagation and dormancy characteristics and introduction of resistance to pest or pathogens (Schierenbeck and Ellstrand, 2009; Mertens, 2008; Tappeser et al., 2014). An increase in fitness of a crop-wild hybrid was reported in wild sunflower hybrids in response to competition and most importantly to the application of the herbicide (Mercer et al., 2007). However, the competitive fitness of susceptible and resistant common cocklebur against acetolactate synthase was not significantly different suggesting that case-by-case risk assessment studies are needed before approval of any GM crop for commercial cultivation (Crooks et al., 2005).

2.1.4. Chemical toxicity

Naturally, plants employ toxins to defend themselves against threats like pests and pathogens. Such chemicals cause toxicity to biotic and abiotic factors of the environment. Toxins such as glycoalkaloids, ricin and delta endotoxins are of greater risk concerns and are extensively investigated. *Bt* delta endotoxins have been targeted in most GM plants and the effect of their proteins on the environment as well as friendly organisms have been studied extensively (Yu et al., 2011). Among the sources of transgenes, bacteria are the most common while, fungi, plants, animals and humans have also been used as sources of various transgenes. Transgenes from these hosts are used either for plant codon usage or for direct molecular evolution (so called molecular breeding) (Keese, 2008; Environmental Risk Management Authority, New Zealand, 2006). Direct gene transfer expresses the desired proteins in the recipient organism while through molecular breeding numerous parental genes are fragmented and reassembled to express novel proteins which are not present in nature. For example, a novel carotenoid was expressed in *Escherichia coli* by shuffling of DNA coding for a pair of enzymes involved in carotenoid biosynthesis pathway (Schmidt-Dannert et al., 2000). So, there exist risks affiliated with natural as well as novel toxins being expressed in the plant body. Risk assessment of natural toxins could be based on certain developed models. However, novel toxins may have target as well as non-target impacts on life. We are concerned with risks from both types of toxins, either natural or novel. Engineered toxins responsible for growth or stress resistance could have unintended effects on ecosystem through certain negative interactions. Environmental impact of toxins responsible for herbicide tolerance and insect/pest resistance is analyzed below.

2.1.4.1. Herbicide toxicity. Herbicide toxicity risks can be considered as a qualitative estimate which includes the possibility and severity of either immediate or delayed adverse effects on the environment, human health and the farmer's economy. However, there are certain factors to which the likelihood and severity of each toxic effect are associated, such as crop and trait, local weed flora, farm management practices and climatic conditions (Madsen et al., 2002). Potential threats to farmland and wild habitats are associated with the cultivation of herbicide-tolerant GM crops. 80% of transgenic crops cultivated at the laboratory or commercial scale have transgenes expressing tolerance to glyphosate, glufosinate and/or stacked with insect resistance. Apart from toxicity to plants themselves, possibility of toxicity to other life forms also exist. Johal and Huber (2009) explained in detail about the direct glyphosate-induced plant defense weakening and increased pathogen virulence. Glyphosate inhibits the plant's defense and structural barriers and immobilizes micronutrients such as manganese (Mn) which play vital roles in disease resistance. Plant nitrogen metabolism is modified in response to applied glyphosate in a similar manner to high temperature-induced modifications. The transient resistance of soybean and wheat rust were reduced when glyphosate treatment modified the nitrogen and carbohydrate metabolism. Certain reports confirmed lethal effects of roundup on amphibians, larval amphibians, fish, tadpoles, snails, insect predators, small arthropods, fungi and bacteria (Relyea, 2005; Morjan et al., 2002). There was almost a complete mortality (96–100%) rate of post-metamorphic amphibians and North American tadpoles in response to direct application of roundup (Relyea, 2005). Even, concentrations below environmental protection agency (EPA) levels harmed Pacific Northwestern Amphibian larval community when exposed to 0–5.0 mg dilutions (King and Wagner, 2010). Herbicide stratification was directly linked to temperature stratification and implicated the habitat choice in ectotherms (Jones et al., 2010). Application of roundup on rice has proven the increase of mortality in water weevil (*Lisorhoptrus oryzophilus*) in terms of 20% reduced larval incidence on herbicide treated rice (Tindall et al., 2004). Liver congestions, necrosis (2.5 to 5.5 times higher) and sever nephropathies (1.3 to 2.3 times higher) was found in male Sprague-Dawley rats fed with roundup applications in drinking water and GM maize diet (DKC 2678 R-tolerant NK603) for two years. The noticeable point is that even lower concentration than field application rates was also tested and found to be of concern. In the case of female rats, mortality increased two to three times and pre-mature death was observed whilst, mammary tumors appeared more frequently (Seralini et al., 2014). Antimicrobial activity of glyphosate and glufosinate is another rising concern (Samsel and Seneff, 2013) as Kruger et al. (2013) clearly stated that glyphosate disrupts intestinal bacteria in cattle and poultry. Some scientists suggested altered defense response of plants against microflora (Benbrook, 2016). Increase in bacterial biomass, enhanced activities of urease, alkaline phosphatase, and invertase have been observed in the rhizosphere of Basta-tolerant oilseed rape grown with the application of Basta (glufosinate) and Butisan S (metazachlor) depicting that GM plants and applied herbicides modify activities of the associated microflora (Sessitsch et al., 2005). Decreased activity of *Bradyrhizobium japonicum* (a nitrogen-fixing bacteria), *Azotobacter chroococcum*, *A. vinelandii* and entomopathogenic bacteria have been reported (Morjan et al., 2002). Such decreased activities of microorganisms especially of nitrogen-fixing bacteria indirectly reduced soybean yield by 8-10% because of inhibition of nodule formation, reduced nodule biomass and reduced nitrogen fixation (King et al., 2001). Effects of herbicide treated fodder on animals as well as rumen microflora can be speculated however, detailed experimental evidences are awaited. Alteration of Cytochrome P450 raised another affiliated risk of glyphosate use. Suppression resulted in a synergistic effect with intestinal bacteria and disrupted aromatic amino acid biosynthesis and could be a pathway to many modern diseases (Samsel and Seneff, 2013). Apart from such effects on other life forms, the health of GM plants itself is another issue. Frequent application of glyphosate could possibly increase the susceptibility of crop plants by increasing the incidence of microflora in the rhizosphere. For example, *Fusarium solani* was reported to have higher incidence after glyphosate application (Njiti et al., 2003). Increased significant of disease severity in terms of weakening of plant defense mechanisms and casual organism population increase is a common hypothesis among plant pathologists. This can be indirectly related to immobilization of micronutrients associated with diseases, hampered growth of the plant, altered physiology and behavioral modifications in soil microflora (Johal and Huber, 2009). Kremer et al. (2005) documented confirmed that microbial components of GM soybean and maize rhizospheres were altered in response to GM crop cultivation and cultural practices. Duke et al., (2012) concluded in a comprehensive review that mineral balance in herbicide tolerant plants is not significantly affected and disease incidence is negligible after using glyphosate and the fact that current amount of evidence in insufficient in this context. Decreased aromatic amino acid levels i.e. phenylalanine and tyrosine in RT crops resulted in decreased effectivity of the plant defense mechanism against abiotic stresses as well as pathogens (Benbrook, 2012). Continuous use of herbicides causes a differential expression of transgenes in specific tissues as in the case of cotton, where reproductive tissues had higher concentrations of glyphosate (Pline et al., 2002). If a plant part with higher accumulation of glyphosate is used for food or feed, it will increase health risks for humans and animals depending upon the part of GM plant to be consumed and expression level of the transgene in that particular plant part. Bohn et al. (2014) investigated compositional differences in GM soybeans and reported high residues of glyphosate and aminomethylphosphonic acid in glyphosate tolerance GM soybeans. Young et al. (2015) presented a detailed report on the role of glyphosate in human endocrine disruption and cytotoxicity to human cells. Again, such increased concentrations will also effect the plant itself regarding pollination problems, decreased pollen viability, boll retention and boll abortion (Pline et al., 2002). Over and above direct toxic effects, indirect effects of herbicide tolerance include disturbed biodiversity of weeds, weed inhabiting arthropods, parasitoids, predators and decomposers which can possibly lead towards disturbance in symbiotic relationships, decreased population of beneficial insects and rapid change in the farmland food chain (Schutte and Schmitz, 2001). Conclusively, the cultivation of GM crops with herbicide resistance, influence host plants as well as non-target soil life, weeds and farmland biodiversity depending upon the degree of adoption. Despite abundant laboratory, green house and farmland scale studies, a considerable knowledge gaps regarding potential induced herbicide toxicity still exist. For more insights about the toxicity of glyphosate based herbicides on mammals please refer to Mesnage et al. (2015). For the coming years glyphosate will prevail as the herbicide of choice across the globe and quantification of its human health impacts and ecological consequences will prosper (Benbrook, 2016).

2.1.4.2. Insecticide toxicity. The most challenging consideration in the development of a resistant GM plant is identifying a resistance gene and directing its product to appropriate plant tissues so that it targets only the pest, without any side effects on friendly organisms. Bt delta endotoxins are the most important examples of engineered insect resistance apart from proteinase inhibitors, α-amylase inhibitors, avidin, chitinases and lectinases (Dale et al., 2002). Previously bacterial formulations with toxin preparations were employed to spray directly to control targeted insects. Preferences were shifted towards expression of toxins in transgenic plants which appeared to be comparatively efficient as well as safe at eliminating insect pests (Schmitz and Schutte, 2001). GM plants produce toxins throughout their life, but sprayed formulations are employed for a particular time. Although the expressed Bt toxins are different from natural toxins and therefore maybe less specific but sprayed natural toxins are rapidly broken down in natural conditions. Commercialization of GM plants expressing Bt toxins was rapidly adopted by the farming community and the area under GM plants is increasing every year, so the ultimate potential target and non-target impacts of transgene expressed toxins are being questioned by a broad community of researchers. Many laboratory scale studies have been conducted to answer the question “Are Bt toxins killing monarch butterflies?” well the answer to the question is contradictory. First laboratory scale report related to mortality of monarch butterfly caterpillars in response to pollen from a commercial Bt maize proved that Bt toxins have a potential hazard to non-target life forms (Losey et al., 1999). This was followed by numerous studies which also came to an agreement with toxicity concerns raised by Losey and coworkers (Obrycki et al., 2001; Stanley-Horn et al., 2001). However, later investigations concluded that toxicity to the host plant as well as non-target species depends on variety of factors such as amount of pollen produced, weather conditions, local fauna and flora, alternative host species for non-target insects, transformation event, promoter, level of expression of toxin, the tissue of GM plant where transgene is being expressed, likelihood of exposure and routes of exposure (Fontes et al., 2002; Hendriksma et al., 2011). There are abundant farm and laboratory scale reports mentioning the hazards of Bt and other toxins on lacewings, earthworms, herbivores, honeybees, human fetus (Saxena and Stotzky, 2000; Agrawal, 2000; Aris and Leblanc, 2011). No significant risks were affiliated with larval survival and prepupal weight of honey bees in response to Bt-maize pollen. Contrary to GM maize, *Heliconia rostrate* pollen posed significant toxic effects (Hendriksma et al., 2011). Delayed growth accompanied by reduced weight gain was witnessed in herbivores feeding on plants expressing sub-lethal Bt doses (Agrawal, 2000). Higher mortality, reduced egg production and a lower proportion of females reaching maturity were observed in *Daphnia magna*; a crustacean arthropod, when fed with Cry1Ab maize (Dekalb 818 YG) (Szenasi et al., 2014). Bt doses could then possibly affect tri-trophic interactions (i.e. plant-herbivores-their natural enemies) in synergistic, additive, or antagonistic ways. Effects of Bt toxins on other trophic-levels including vertebrate predators preying on lepidopteran pests are yet to be considered (Clark et al., 2005). The presence of Bt toxins in aphid (*Myzus persicae*) samples detected by a double sandwich enzyme-linked immunosorbent assay, confirmed possible consequences of these toxins in food chains and trophic levels of herbivore-natural enemies (Burgio et al., 2007). On the contrary, many researchers reported no toxicity to non-target species because of shorter persistence or degradation of Bt toxins in the soil (Saxena and Stotzky, 2000; Oraby et al., 2015; Kroghsbo et al., 2008; Domingo, 2000). Recently, toxic effects of Bt toxins on non-target soil organisms as well as insects was reviewed and concluded in opposition to significant non-target risks (Yaqoob et al., 2016). However, non-inertness of combined effect of Cry1Ab and Cry1Acas well as in response to 1 to 200,000 ppm was confirmed. Cry1Ab concentration of 100 ppm resulted in the death of human embryonic kidney cells (Mesnage et al., 2012). Domingo (2000, 2007, 2011b, 2016, Domingo and Giné Bordonaba, 2011) reviewed adverse health effects of GM crops and summed up the published studies and reported the health effects of GM crops are same as their counter parts with few exceptions. Which clearly suggests that the presence of controversial experimental results it is hard to consider GM food and feed safe.

Considering the chemical toxicity, we can conclude that most of the chronic and sub-chronic studies that have been done till now for testing the toxicity of genetically modified (GM) organisms used as food and feed lack to show any potential health effects (Domingo, 2016), but have a lot of limitations such as the period of exposure, that is too short to evaluate the long-term effects and the endpoints that are limited and not cover the whole area of testing (Hilbeck et al., 2015) as neurodegenerative toxicity, mutagenicity, teratogenicity and carcinogenicity (Hernandez and Tsatsakis, 2017; Tsatsakis et al., 2016a). The classical toxicological studies don't take in considerations the hole area of interactions that could appear in real life exposure between GM organisms and other chemicals that humans are exposed every day, even at doses below or around regulatory limits that could lead to a synergistic and potentiation effects (Hernandez et al., 2013). Also, these types of single compounds studies don't focus on different types of long-term toxicity for which at present there is a special concern regarding neurotoxicity, cardiotoxicity, nephrotoxicity, genotoxicity, hepatotoxicity and endocrine disruption. It is a fact that the international regulatory authorities have also started to realize the need for cumulative risk assessment and new methodologies are being developed, but only for commercial artificial mixtures (EFSA journal, 2013; Regulation 1272/2008/EC, 2015). No regulatory provision has been taken for non-commercial artificial mixtures that represent the real scenario of real life exposure. As for pesticides and other chemicals to which consumer are exposed over the lifetime, also for GM organisms there is a need to pass from single compound risk assessment to cumulative risk assessments that hazard the long-term exposure to low doses of chemical mixtures monitoring different endpoints at the same time associated also with investigated the systemic mechanistic pathways like oxidative stress (Tsatsakis et al., 2016a, 2016b; Tsatsakis and Lash, 2017; Docea et al., 2016).

2.2. Indirect impact of transgenes on environment

The impact of transgenic crops on the environment is obvious in response to changes and modifications to current agronomic practices or broadly speaking agricultural practices. Indirect impacts of GM crops include effects on soil, water, wildlife biodiversity and reduced efficiency of weed, insect and pest control. The extent of a risk is mainly dependent on the nature of changes in agricultural practices (ICSU, GM Science Review Panel). Still, it needs to be decided whether the overall impact of such modified use of pesticide has positive or negative prospects, but reports exist which pave the concept that changing agricultural practices have disturbed habitats of farmland fauna and flora.

2.2.1. Effect on soil and water

Continuous debate still prevails among scientists and farming communities about the effects of GM crop introduction on ground water and water reservoirs. This debate is directly related to the extent and amount of herbicide use on GM crops. As it is known, GM crops are tolerant to herbicides and invite broad-spectrum herbicide applications (Benbrook, 2012). This increase in herbicide use was indirect i.e. replacement of more toxic herbicide which persists more in the environment with glyphosate (Duke et al., 2012). In a sense, there is an overall decrease in the application of tones of toxic herbicides and an increase in glyphosate-based herbicides is noticed (Benbrook, 2016). Glyphosate is probably the most widely used herbicide in the world. Glyphosate can reach the soil from the direct interception of spray during early season or post-harvest applications, from run-off or leaching of the herbicide from vegetation and by exudation from roots or death and decomposition of plant material (Duke et al., 2012; Kremer et al., 2005). The addition of glyphosate in farmland water and ultimately to the aquatic ecosystems and its impact on aquatic life is apparent. However, the risk of glyphosate toxicity to non-target soil biota is often considered to be marginal owing to a shorter half-life compared with many other herbicides and strong adsorption to the soil matrix. Zabaloy et al., (2016) showed no negative effects on soil microbial communities in fields that were exposed to glyphosate. This study suggests that glyphosate use at recommended rates poses a low risk to microbiota (Duke et al., 2012; Borggaard and Gimsing, 2008). The antimicrobial activity of glyphosate is a matter of debate too, because large scale applications of glyphosate would certainly disturb microbial communities at farm scale (Samsel and Seneff, 2013).

Concomitantly, transfer of Bt toxins from GM crops to soil and water have many possible routes including pollen deposition during anthesis, root exudates, and GM plant residues (Yu et al., 2011). Evidence exists that Bt toxins bind to the clay and humic substances, rendering the proteins biodegradable (Clark et al., 2005; Saxena and Stotzky, 2000). Once the protein is bound to the clay particles their susceptibility to degradation decreases as observed by Stotzky (2004) with special reference of Cry1Ab, Cry1Ac, and Cry3A in root exudates of GM maize, potato, rice, canola and cotton. But the un-intended effects of these proteins on soil residing organisms were not consistent and were not taken up by roots of non-GM plants. Statistically, non-significant pH levels under Cry1Fa2 GM maize were observed as compared to soils under non-GM maize (Liu et al., 2010). Most studies have suggested that Bt proteins from transgenic plants break down relatively rapidly in the early stage after entering the soil and that only a small amount of them can remain for a long time period, so that Bt proteins do not bio-accumulate in soil (Rauschen et al., 2008; Yu et al., 2011). However, the persistence of Bt toxins in the soil is largely dependent on the type of toxin and soil type not the number of expressed transgenes (Rauschen et al., 2008). As a result of less chemical pesticide being sprayed on cotton, demonstrable health benefits for farm workers have been documented in China (Pray et al., 2001) and South Africa (Bennett et al., 2003).

2.2.2. Effect on biodiversity

Widespread commercial cultivation of GM crops especially herbicide tolerant crops pose serious threats to the ecosystem complexity and reduction in biodiversity. Contrary to yield loss and contamination, weeds are ecofriendly in a sense too; consider the reduction of soil erosion by weeds and provision of habitat to a range of beneficial organisms (Mertens, 2008). Likewise, studies showed that the diversity, density, and biomass of the seed bank in farmland were evidently lesser in GM systems contrary to conventional systems (Bohan et al., 2005). UK Farm Scale Evaluations (FSE) reported 20–36% reduction in weed seed bank (Andow, 2003). However, the report discovered that dicot weeds were more susceptible than monocots). Rapid changes in habitat destruction will broadly impact changes in food webs and food supplies. So, the balance of the predator-prey systems becomes even more critical besides the impact on beneficial organisms. This will not end up here, of course, disturbed tri-trophic interactions and symbiotic associations will also be the consequence leading to complicated disturbance in the food web. It is obvious that such disturbance in weed, insect and pest management will, in turn, end up with increased use of pesticides (Schutte and Schmitz, 2001). This change in resource accessibility, pose knock-on effects on higher trophic levels in most cases. Foraging behavior can also be modified by the frequent application of herbicides as in the case of glyphosate application where spiders moved to superfluous cricket killing behavior (Marchetti, 2014). Other consequences involve shifting in the food web (e.g. from herbivore to detritivore). Short term shift in soil biota have been witnessed in farmlands where glyphosate tolerant maize and soybean were planted Application of glyphosate resulted in increased fungal biomass in relation to bacterial biomass which paves the hypothesis of a shift in the food web on the basis of slower nutrient turnover and harnessed enrichments; based on resources of the carbon and nitrogen ratio (C:N ratio) (Powell et al., 2009). The factors involved in disturbance of farmland biodiversity include types of herbicides and insecticides used, degree of adoption, frequency of application, timing of herbicide or insecticide application, target crop, rotational and agronomic practices adopted, local fauna and flora, alternate hosts for friendly insects, microclimatic conditions, management history and surrounding habitats (Mertens, 2008). Emigration of the agrobiont wolf spider (*Pardosa milvina*) was reduced when observed under the application of Baccaneer® Plus (glyphosate) indicating that there exists a disturbance in a predator-prey relationship in food webs across the eastern US (Wrinn et al., 2012). Parallel to herbicide tolerant GM crops, Bt crops have been questioned too for their possible threats to biodiversity. Pesticides are often transported beyond crop fields and can show considerable impacts on terrestrial and aquatic ecosystems or on plant populations in the vicinity of crop fields. Most prominent targets are mammals and birds and many studies have revealed little or no evidence of Bt toxicity to these animals (Flachowsky et al., 2005b; Aris and Leblanc, 2011). To a broader sense, it could be concluded that biodiversity is negatively affected by the cultivation of HR GM crops (Bohan et al., 2005; Isenring, 2010; Lovei et al., 2010). The discussed dangers to biodiversity could be possibly observed on a long term basis and of course, risks could not be left out of the equation. However, one short-term food web assessment (a two-year investigation) in response to the cultivation of GM maize revealed the presence of stable and complex food webs and their persistence was not compromised. The study included GM maize having resistance against Coleoptera, Lepidoptera and glyphosate and mainly focused on arthropod food webs with an experimental population of 243,896 individuals (Szenasi et al., 2014).

2.2.3. Reduced efficiency of pest, disease and weed control

Frequent crop swooping, increased use of broad-spectrum herbicides and increased impetus on minimal cultivation/zero-tillage agriculture systems are few of the consequences of changes in agronomic practices in response to GM crop introduction (Dale et al., 2002; Tappeser et al., 2014). Of course, many advantages are associated with changed agricultural practices such as soil erosion, less disturbance to earthworms and minimal disturbance to soil microclimate especially in the case of zero tillage. Contrarily, many indirect risks are associated too, such as the evolution of RT weeds, weed population shift, development of cross-resistance and multiple resistance and evolution of resistance to Bt toxins. We mainly focus on the evolution of herbicide and insecticide resistance.

2.2.3.1. Evolution of herbicide resistance. The appearance of RT weeds is inevitable with the fact that weed species harbor a remarkable ability to evolve herbicide tolerance from within the weed gene pool (Agapito-Tenfen et al., 2014). Weeds can evolve herbicide resistance in about three years as cases of polygenic herbicide resistance had been reported in horseweed for F1, F2 and backcross progenies when exposed to low doses of diclofop-methyl (Busi et al., 2013). Increased prominence of Asiatic dayflower (*Commelina cumminus* L), wild buckwheat (*Polygonum convolvulus* L) and common lambsquarters (*Chenopodium albus* L) was observed where significant selective pressure was present due to concomitant use of herbicide and frequent cultivation of herbicide-resistant crops (Owen and Zelaya, 2005). In tolerance development, various mechanisms could help the plant such as target site over production, modification in intracellular herbicide compartmentation, minimal herbicide absorbance and translocation, herbicide detoxification and insensitivity to target site (Brower et al., 2012; Velkov et al., 2005). Although the probability of target-site resistance to a single herbicide is quite low but not negligible i.e. one individual in 10^{-5} to 10^{-10} while the frequency is almost half when multiple-target site resistance is considered (Mortensen et al., 2012). As of February 2016, a total of 467 unique cases of RT weeds have been recorded globally belonging to 249 species (144 dicots and 105 monocots). These 249 species are resistant to 22 of the 25 known herbicide sites of action and to 160 different other herbicides (http://www.weedscience.org). Glyphosate and glufosinate resistance from within the weed gene pool is highly unlikely mainly because of its chemical structure, no residual activity, limited glyphosate uptake by plant roots from the soil, mode of action and near zero soil persistence (Baylis, 2000). Few reports of glyphosate resistance development are annual rye grass in Australia and horseweed in the US (Dale et al., 2002). This resistance development might be overexpression of target enzyme, reduced herbicide translocation and different sensitivity of target enzyme to glyphosate (Wakelin et al., 2004). On a large geographical scale, many independent evolutionary events could simultaneously interplay for the emergence of herbicide resistance (Bonny, 2016). Regular use of glyphosate on a considerable proportion of GM crop fields make the assumption of glyphosate resistance development a reasonable hypothesis. It is not mandatory for weeds to be a poorer competitor than susceptible weeds as no fitness differential was detectable between susceptible and resistant biotypes of *Lolium rigidum* (Busi et al., 2013). Conclusively, although the evolution of resistant weed biotypes, development of cross and multiple resistance and weed population shift is inevitable, evolution delay strategies could though comprehend the herbicide resistance development (Schutte and Schmitz, 2001).

2.2.3.2. Evolution of insecticide and pesticide resistance. Controlling pests through conventional and chemical techniques have been proven to be challenging as evolution of insecticide and pesticide resistance has been witnessed in many cases (Dale et al., 2002). More specifically, the possibility of evolution of Bt-resistant insect pests can't be negated because of constitutive expression of Bt toxins in all plant tissue imparts higher selection pressure on target species (Yu et al., 2011). Use of Bt bio-pesticides by organic farmers lead to resistant diamondback moth populations in Central America, Florida, Japan, Philippines, Hawai, and China (Tabashnik et al., 2005, 2013). Gassmann et al., (2014) reported that Bt corn with higher toxin dose offers higher selection pressure to western corn rootworm which has resulted in the development of cross-resistance between Cry3Bb1 maize and mCry3A maize. Also, many laboratory scale studies reported the selection of resistance to Bt toxins European corn borer, pink bollworm, cotton bollworms (Zhao et al., 2001; Akhurst et al., 2000; Burd et al., 2003). The intensity of selection is a major driving force in determining the rate of resistance evolution along with size and arrangement of refuges, mating behavior of insect pest, seasonal changes in habitat and population regulation by insecticides in GM crop and refuges (Caprio, 2001). Mechanism of resistance development is variable among target insect pests such as prevention of midgut toxin binding to the intestine, overexpression of toxin, intestine proteases with altered activities, inhibition of cell lysis in response to ionic compensation mechanisms and modification in the toxin-receptor complex (Akhurst et al., 2000). A decade back report by Tabashnik et al. (2004, 2005) explained the presence of recessive alleles of the cadherin (BtR) gene in the resistant strains of pink bollworm (*Pectinophora gossypiella*) that were associated with resistance to Cry1Ac. In nematodes a different resistance mechanism was reported by Griffitts et al. (2001) and lack of the protein encoded by bre-5 (a putative ß-1, 3-galactosyltransferase) in the *Caenorhabditis elegans* intestine resulted in no binding leading to resistance to the Bt toxin Cry5B. Tabashnik et al. (2013) surveyed 77 reports claiming evolution of pest resistance to Bt toxins from five continents and confirmed field-evolved Bt toxin resistance in 5 of 13 species under discussions. Two strategies for the delay in resistance evolution has been proposed by EPA i.e. high toxin dose and high dose refuge (www3.epa.gov). Gene pyramiding strategy has also been proposed by Dale et al. (2002) which delays the evolution of resistance in a much more effective way. Contrary to resistance development against Bt toxins and insecticides, pathogen resistance development is quite high because viruses, bacteria, and fungi are known to adapt very rapidly to selective forces. Principally, single gene based resistance mechanisms are easy to overcome. Also, frequent mutations in avirulence (Avr) genes of bacteria and fungi have been reported, so resistance mediated by the integration of the corresponding resistance (R) gene can be overcome. Other field based evolution delaying strategies include low initial frequency of resistance alleles, recessive inheritance, abundant refuge populations and deploying two-toxin Bt crops rather than single-toxin Bt crop (Tabashnik et al., 2013).

3. Science and politics in regulation of GMOs

3.1. Science and politics in EU regulation of GMOs

The predominant place of science in EU Risk Regulation and especially in the decision-making procedure for the authorization of GMOs is indisputable. For instance, the first substantive stage in the above-mentioned procedures is the scientific opinion from the European Food Safety Authority (EFSA). Commission decisions on authorization are based overwhelmingly on EFSA scientific opinions (Lee, 2014). These opinions are the EFSA's review of the risk assessment carried out by the applicant for authorization. Pursuant to Article 3(11) of Regulation 178/2002 (www.eur-lex.europa.eu) risk assessment is defined as “a scientifically based process consisting of four steps: hazard identification, hazard characterization, exposure assessment and risk characterization”. Moreover, the risk assessment, pursuant to Article 6(2) (www.eur-lex.europa.eu) of this regulation, “shall be based on the available scientific evidence and undertaken in an independent, objective and transparent manner”.

However, the risk assessment has important limitations because it is pervaded by key uncertainties surrounding the dose-response curve, the applicability of results from animal and *in vitro* studies to humans or extrapolations from a high level of exposure in controlled environment to lower levels encountered usually outside of the laboratory (McGarity, 2001). Besides these limitations, the objectivity of science is socially constricted because it is better understood not as an intrinsic attribute of science but as a perceived characteristic of scientific knowledge, arrived through culturally conditioned practices (Jasanoff, 2015).

Moreover, it should be noted that the undisputed uncertainties undermine the value of the generated results of risk assessment so that it does not accurately describe the health and environmental risks. In particular, doubts about the safety of GM foods have been raised as scientists frequently disagree about the interpretations and inferences drawn from a study (Krimsky, 2015). In this situation of uncertainties, ignorance, and doubt, policy plays a role in the competent authority’s choice among conflicting interpretations and, consequently, the decision taken is based not only on scientific knowledge but on societal and cultural values as well. Thus, the interplay between science and politics is an intrinsic attribute of EU regulation of GMOs.

However, against this institutional background EFSA pays little attention on the uncertainties, assuming that risk assessment can capture risks with sufficient certainty and reasonable accuracy. Despite the fact that EFSA indulges in the existence of scientific uncertainty (for example the opinions concerning the maize 1507 and potato Amflora), the basis of scientific assessment for which there have been strong objections remains the same. Specifically, EFSA has published guidance documents concerning the carrying out of Environmental Risk Assessment (ERA) as it is prescribed in the Directive 2001/18 and the Regulation 1829/2003 (EFSA, 2010, 2012, 2013). Those guidance documents are not legally binding but they matter greatly because they are documents which have been drafted by EFSA, the official authority which carries out the review of the ERA. Although many points concerning the carrying out of ERA are clarified, one point of EFSA’s guidance document causes serious concerns (ENSSER, 2011; Fagan et al., 2014). It is the comparative assessment as a new principle of ERA. The comparative safety assessment is nothing more than the known principle of “substantial equivalence” on which the risk assessments about the GMOs pursuant to the USA Statement of Policy (1992) is based. This particular principle which has been expressed for the very first time by Organization for Economic Co-operation and Development, its start line is the admission that the history of a safe use of a conventional plant may be the basic parameter for the risk assessment of GM plants which derives from the counterpart conventional plant(s) (Kok and Kuiper, 2003).

Despite its wide use, the substantial equivalence principle has been considered as a non-scientific principle and as a political choice which is directed by industries (Domingo 2000, 2007, Domingo and Giné Bordonaba, 2011a). For these reasons, it has been widely criticized. For example, it has been considered that it is deficient and partial because it is based only on chemical similarity without taking into account the biological, toxicological and immunological data (Kysar, 2004; Bratspies, 2007). Furthermore, between the obstacles for a complete comparison between the GMO and the counterpart conventional plant is the lacking knowledge of basic factors as the toxicity’s levels of antinutritional factors in less economically important plants, the different and various environmental conditions as well as the difficulty in tracking the appropriate conventional plant to be compared with the GM plant.

Given the pervasive uncertainty about GMOs safety, as noted earlier, EFSA and the European Commission need to acknowledge two things, one being the existence of limitations about scientific information and the other being the relevance of the precautionary principle during the risk assessment and risk management stages (Skogstad, 2011). It is important to note that the EU legislation explicitly calls upon the competent authorities to take into account, in the authorization process, not only the scientific evidence but also other legitimate reasons. So, the obligations to justify their decisions on scientific grounds and to take other factors into account are actually complementary.

3.2. Science and politics in the Russian Federation GMO regulation

Nowadays the use of up-to-date technologies, in particular, biotechnology, in the domain of providing country food supply, appears to be the one of state priorities in the Russian Federation (Tyshko and Sadykova, 2016). In order to execute this actual regulatory and methodical base was created which can been observed in federal laws i.e. “On the state regulation in the sphere of genetic engineering activities” (No 86-FZ from 05.07.1996), “On the sanitary and epidemiological public wellbeing” (No 52-FZ from 30.03.1999), “On the quality and safety of food products” (No 29-FZ from 02.01.2000), “On the protection of consumers rights” (No 2300-1 from 07.02.1992), and "On amendments to certain legislative acts of the Russian Federation to improve state regulation of genetic engineering activity"(No 358-FZ from 03.07.2016). Apart from these laws many decrees of the president of the Russian Federation aid in regulation such as decree of the president of the Russian Federation No 120 from January 30, 2010, “On approval of food security doctrine of the Russian Federation”, decree of the president of the Russian Federation No 899 from July 7, 2011, and “On approval of the priority directions of science and technology development in the Russian Federation and the list of critical technologies of the Russian Federation”.

Many resolutions approved by Russian government also emphasis on safety regulations related to foods developed through biotechnology e.g. resolution of the Russian Government No 717 from July 14, 2012, “On the state program for development of agriculture and regulation of agricultural and food markets in 2013–2020”, “A comprehensive program of biotechnology development in the Russian Federation for the period till 2020”, approved by Government of the Russian Federation No 1853p-P8 from April 24, 2012, resolution of the Russian Government No 839 from September 23, 2013, “On the state registration of genetically-engineered-modified organisms intended for release into the environment as well as products derived with the use of such organisms or containing such organisms” and resolution of the Russian Government No 548 from June 16, 2014, “On the amendments to the Resolution No 839 from September 23, 2013”.

The most advanced system of GMO regulation in the Russian Federation is the system of state registration of plant GMOs which are intended for use in food. The devise of GMO of plant origin safety assessment system, which is currently valid in the Russian Federation, was initiated in 1995–1996. Not only does the system accumulate all domestic and foreign experience, it also includes the latest scientific approaches, based on achievements of fundamental science (Tyshko et al., 2007). GMO safety assessment is carried out at the stage of state registration. The subject of registration is novel food products derived from GMO, manufactured in the Russian Federation, as well as food products, entering the Russian Federation for the first time. In the system of medical and biological assessment of the GMO safety, along with general toxicological research, the study of specific types of toxicity in *in vivo* experiments takes a prominent place. In accordance with established research practice, which uses an integrated approach, and provides with the most complete and reliable information on potential reprotoxic, genotoxic, immunotoxic and allergenic effects of GMO, as well as enables to reveal possible unintended effects of genetic modification (Tutelyan et al., 2009, 2010; Tyshko et al., 2009, 2011, 2014). From 1999 to 2017, 23 GM lines passed the entire cycle of medical-biological investigations. By 2017 there had been amassed scientific foundation on GMO safety, which includes the analysis of result of research, carried out within the framework of GMO state registration in the Russian Federation, as well as domestic and international data, presented by scientific literature on the problem of biotechnological food safety, at both registration stage and post-registration monitoring stage. To sum up, in the Russian Federation the most essential issues, regarding the use GMO for food purpose have been elaborated: (1) legislation, normative and methodical foundations, regulating safety assessment and control over GMO turnover, as well as ensuring customer awareness about presence of GMO in food products, have been created; (2) so far, the existing experimental evidence *in vivo* in rats reports on the lack of negative toxicity effects i.e. GMO safety has been assumed; (3) the possibility of control over GMO turnover throughout the food market of the country has been sustained.

4. Future perspectives

In response to the commercialization of GM crops across the globe, questions are being raised whether long lasting effects are good or bad? The amount of uncertainty and unpredictability of risks associated with GM crops developed through modern biotechnological techniques or conventional mutation breeding or hybridization is variable but sure. Considerable increase in area cultivated under GM crops engineered with a single gene or stacked traits has been witnessed since the last three decades. It is important to examine the variable potential risk of GM crops within the context of wider knowledge and case-to-case basis. (i) To avoid harm to beneficial organisms, spatial and temporal expression of transgene should be considered to focus mainly on plant tissues where the resistance mechanism is exactly needed. For this, a variety of promotor systems or so-called tissue-specific promotors should be identified and examined. Laboratory scale studies should be conducted to understand the type of risk and its actual potential in a broader environment or farmland systems. (ii) Case-by-case assessment should be done to make universal judgments targeted to inescapable or invasiveness of transgenes or wild × GMO hybrids and their progenies. Plant and seed characteristics which could help in improved survival should be considered along with modification in breeding systems. (iii) Large-scale investigations are required to identify possible hosts for gene transfer. Possible consequences of single of stacked gene flow should also be assessed. The assessment could also be based on the nature of transgenic trait. (iv) The avoidance of the narrow genetic base of resistance is another strategy to minimize the risk. For this, diverse disease resistance and tolerance mechanisms should be discovered and employed as rather similar mechanisms in a variety of crops. (v) Cheaper gene stacks and marker genes should be adopted which in most cases are the previously registered stacks. Such stacks will avoid regulatory cost and time for registering. In the case of stacking care, it should be taken into account so as to avoid new deadly toxin combinations having adjuvant and synergistic effects. Next generation sequencing and hundreds of genome sequenced artificial regulators could be developed having well characterized cis-regulatory elements, insulators, and enhancers with predicted features and reduced risks. Other features of genomes such as epigenetics, DNA methylation, histone modification and microRNAs should also be considered prior to designing a stack. (vi) Many of transgenes are already present in the environment so studies should be conducted to investigate the comparative survival of identical genes in a GM crop. (vii) It should also be determined whether the foreign DNA could retain post-transformation integrity or not? (viii) Possible routes of gene flow through competent bacteria or viruses should be considered. For this, laboratory-scale investigations will be able to determine whether particular transgene would flow through soil microorganisms or not and if in the situation it will flow, what will be the possible risk. (ix) Complex food webs and food supplies should be considered case-by-case before the release of new transgene harboring resistance traits. (x) Mono-crop GM culture over the vast areas is another concern with an increasing possible risk. To deal with this problem multi-crop system with a rotation should be implemented in the particular area. (xi) The risk level for insertional mutagenic effects should also be accounted for creating genetic changes in plant genomes. (xii) Transgenes have the ability to enhance the recipient's interspecific competitiveness, bring sever decrease in friendly herbivores population and can invade expanded niche range. For this situation, knowledge about the genetic structure and demography of recipient populations is a basic requirement. (xiii) Quantification of hybridization or outcrossing potential and spatial distribution of cultivated, wild and weed type relatives should be included in pre-release assessments to warrant migration measures. (xiv) Alterations in energetic homeostasis in response to stacked transgene expression suggests an inclusion of omics analysis as a desirable benchmark in risk assessment studies. (xv) While, analyzing novel expressed proteins or unintentionally expressed proteins, their allergic and toxic effect could be predicted using bioinformatics tools. (xvi) Preferable assessment approach for non-target species falling within Bt specificity, the theoretical worst-case scenario test is recommended and advanced exploration of combined effects of Bt proteins is advised. (xvii) Current regulatory pathological and toxicological tests are confined on only one mammal and for 90 days which is insufficient and could not be generalized. So, toxicology studies should be prolonged to full life span of the test organism and other experimental mammals should also be considered for such tests. (xviii) The flow of elements of transgene constructs, such as promoter and terminator, marker genes as well as non-transgenes of a host having abilities to hitchhike along with target transgenes. (xix) Research should be undertaken to discover intra/extra-cellular pathways supporting DNA release from various organisms. (xx) Safety studies involving GM plants produced as a result of ds-RNA mediated gene silencing should be conducted and after effects of administering GM foods having artificial siRNAs must be examined on a laboratory scale especially for their unintended effects on humans. (xxi) Sub-lethal effects on non-target species should be assessed for several successive generations rather than single or two generations. Authors pledge regulatory agencies and farming communities to combine improved agronomic practices and GM crops to reduce eco-toxicological impacts of GM crops on biodiversity, soil, and water, wildlife, fauna and flora. This review, in turn, will help authorities to comprehend risks and give follow-up evaluations and management stratagems. Furthermore, it will raise public as well as farmer's awareness regarding threats from GM crops.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.03.011.

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