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Herbicide resistant biotech crops and their import to Indian agriculture

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ABSTRACT

Herbicide resistant (HR) biotech crops which include both the transgenic and non-transgenic ones are being grown in several countries for over 24 yr. Transgenic biotech crops are derived when an exogenous herbicide-resistant gene/s from non-plant sources is/are inserted into the desired crop plant. When the inserted genes stably integrate and express in the plant genome, the concerned plant behaves like a normal plant but with the acquired character, *i.e.* herbicide resistance. On the other hand, the non-transgenic biotech crops are generated for some herbicides (ALS-inhibiting and ACCase-inhibiting cyclohexane-diones) by selecting for target mutations in plant populations or by tissue culture or by mutation breeding. HR varieties have been developed for soybean, maize, cotton, canola, wheat, rice, sugar beet, alfalfa, *etc.* while the herbicides included glufosinate, dicamba, 2,4-D, phenmedipham, paraquat, imidazolinones, mesotrione, sulfonylureas, *etc.*

About 190 million ha around the world have been under HR transgenic crops in 2017. Around 80% of this area was under HR ones either alone or stacked with insect resistance. Biotech crops have made a positive contribution to global crop production and the economies of farmers, while they certainly raised concerns about biosafety to consumers. Several countries led by USA have widely adopted HR biotech crops, while India has been growing only the insect-resistant (IR) Bt cotton since 2002. With adoption of Bt varieties, the country has achieved a great stride in cotton production, accounting for a quarter of market share in global cotton production in 2017. Although no HR biotech crop is adopted in India, it is grown illegally by farmers in key cotton-growing states. The concerns and limitations about HR biotech crops are related to agro-ecology, evolution of herbicide-resistant weeds, food safety, soil ecosystem, coexistence of biotech and conventional crops, socio-economic consequences, coexistence of biotech and conventional food products, etc. This paper also discusses management of HR biotech crops in greater detail.

Herbicide resistant biotech crops

Biotech crops are designed to become resistant (tolerant) to specific broad-spectrum herbicides which kill the surrounding weeds, but leave the cultivated crop unaffected. Resistance is imparted into the crop by changing the genetic makeup of crop plants. In this process, known as genetic engineering, one or more traits that are not already present are introduced. It involves the use of laboratory tools to insert, alter or cut out pieces of DNA that contain one or more genes of interest. Genes are molecules of DNA that code for distinct traits or characteristics. Biotech crops, also referred as genetically modified (GM) crops or genetically engineered (GE) crops, are of two types: transgenic and non-transgenic.

Transgenic biotech crops

The process of transferring an exogenous gene, called transgene, is referred to as transgenic engineering or transgenesis. When this new gene is inserted, the plant will exhibit a new property and transmit that property to its offspring. Once inserted, transgenes behave like normal plant genes if they are stably integrated and expressed. This genetic engineering technology changes the phenotype of an organism.

Once a transgenic plant is created, the transgenes can be inherited along with the rest of the plant's genes through normal mating by pollination. The offspring are also transgenic when they acquire the transgenes this way. Plant breeders can take a transgenic plant made in the laboratory and use conventional breeding methods to develop different transgenic varieties of the crop that are adapted for specific uses.

Genetic engineering allows the direct transfer of one or just a few genes of interest, between either closely or distantly related organisms to obtain the desired agronomic trait. Not all genetic engineering techniques involve inserting DNA from other organisms. Plants may also be modified by removing or switching off their own specific genes by using 'recombinant technology.' It is a laboratory gene-splicing procedure in which the DNA of the donor organism is cut into pieces using restriction enzymes followed by insertion of one of these fragments into the DNA of the host plant. Using recombinant DNA (rDNA) technology, we can isolate and clone single copy of a gene or an rDNA fragment into an indefinite number of copies, all identical. This technology allows bringing together genetic material from multiple sources, creating sequences that would not otherwise be found in biological organisms. Most of the time, a bacterial or virus plasmid is used to insert the donor DNA.

The process of transgenic engineering requires the successful completion of, a) locating and identifying genes of interest, b) isolation and extraction of DNA, c) cloning, designing and constructing the gene of interest for plant infiltration, d) transformation and e) testing and plant breed-back crossing (Rao 2014, 2018).

Once a new transgenic crop variety is developed, it needs to be assessed in terms of food and safety to the environment. This is carried out in conjunction with testing of plant performance. In this phase, the transgenic varieties need to be assessed for altered nutrient levels, known toxicants, new substances, antibiotic resistance markers, non-pathogenicity to animals and humans, toxicity to non-target organisms, stable integration of the introduced gene(s) in the plant's chromosomes, risk of creating new plant viruses, effects on plant biology and ecosystem, spread of the transgene to other crops and wild relatives, allergenicity, *etc*.

Transgenic herbicide-resistant crop events/ Varieties

Several transgenic herbicide resistant crop varieties/events have been developed since 1994. It was that year which saw the commercial release of the first herbicide-resistant transgenic crop variety, BXN cotton line, developed by CalGene and Rhône-Poulenc. The same year also witnessed the release of the first glyphosate-resistant (GR) crop variety, MON4030-2-6 (GTS 40 3 2) of soybean. In 2005, this event was grown on approximately 87% of the U.S. acreage and 60% of the global acreage under soybean crop (USDA-NASS 2005). Later, another GR event, MON89788, was made available in 2007 and several other countries by 2010. It provided farmers flexibility, simplicity and cost-effective weed control options. These two 'Roundup Ready Soybean' varieties transformed global soybean production significantly.

Since then, scores of herbicide-resistant transgenic crop varieties have been developed for several herbicides. These crops included soybean, maize, cotton, canola, wheat, rice, sugar beet, alfalfa (lucerne), etc. while those of herbicides were glyphosate, glufosinate, dicamba, 2,4-D, phenmedipham, paraquat, imidazolinones, mesotrione, sulfonylureas, etc.

Some of the transgenic crop lines resistant to different herbicides are presented in **Tables 1, 2 and 3**. As it is impossible to include all the events and stacks developed in the world thus far, only a few selected ones are included here.

Gene stacking

Crops are also engineered or "stacked" to express multiple traits to enable them become resistant to multiple herbicides or to herbicides and insecticides together. In this stacking (pyramiding) process, two or more genes (traits) of interest with different modes (sites) of action are inserted into a single plant. An example of a stack is a plant transformed with two genes (e.g., glyphosate-resistant and glufosinate-resistant; glyphosate-resistant and dicamba-resistant) or more that code for proteins having different modes of action. It is a hybrid plant expressing both herbicide resistant genes derived from two parent plants. For example, this is done by combining glyphosate resistance gene *epsps* with the *pat* gene to confer resistance to glufosinate and/or with *dmo* gene to confer resistance to dicamba.

Biotech stacks are engineered to broaden weed control efficiency as also to have better chances of overcoming other myriad of problems in the field such as diseases, abiotic stresses, etc. so that farmers can increase crop productivity. Some of the stacked varieties and hybrids are presented in **Table 4**.

Table 1. Transgenic crop events/varieties developed for glyphosate resistance from 1994

Crop	Event/Variety	Gene(s)	Developer	First Approval (Yr)
Soybean	MON04030-2-6 (GTS 40 3 2)	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	1994
	MON89788	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2007
Maize	GA21	*mepsps*	Monsanto	1997
	NK603 (603)	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2000
	MON832	*cp4 epsps* (*aroA*:*CP4*) *goxv247*		
	MON87427	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2012
	HCEM485	*2mepsps*	Stine Seed Farm	2012
	VCO-01981-5	*epsps grg23ace5*	Genective S.A.	2013
Cotton	MON1445	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	1995
	MON 88913	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2005
	GHB614	*2mepsps*	Bayer CropScience	2009
Canola	GT73 (RT73)	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	1995
	GT200 (RT200)	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2002
	MON88302	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2012
	73496	*gat4621*	DuPont (Pioneer)	2012
Wheat	MON71800	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2004
Sugar beet	GTSB77	*cp4 epsps* (*aroA*:*CP4*)	Novartis/ Monsanto	1998
	H7-1	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2004
Alfalfa (lucerne)	J101	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2010
	J163	*cp4 epsps* (*aroA*:*CP4*)	Monsanto	2004

Genes: *aroA:CP4*: *Agrobacterium tumefaciens* strain CP4; *cp4 epsps*: gene which is the herbicide tolerant form of 5-enolpyruvylshikimate-3-phosphatesynthase; *cp4 epsps (aroA:CP4)*: this gene is called by either name; *epsps grg23ace5*: synthetic gene, similar to natural *epsps grg23* gene from soil bacterium *Arthrobacter globiformis*; *mepsps*: modified 5-enolpyruvylshikimate; *2mepsps*: double mutant version of 5-enolpyruvylshikimate; *gat4621*: glyphosate *N*-acetyltransferase (*gat*) gene derived from *Bacillus licheniformis*; *goxv247*: this gene, derived from *Ochrobactrum anthropic* stain LBAA, produces a modified enzyme GOX that catalyzes glyphosate into aminomethylphosphonic acid and glyoxylate.

Table 2. Transgenic crop events/varieties developed for glufosinate (phosphinothricin) resistance

Crop	Event/Variety	Gene(s)	Developer	First Approval (Yr)
Soybean	GU262	*bar*	Bayer CropScience	1995 (U.S.)
	W62, W98	*pat*	Bayer CropScience	1995 (U.S.)
	A2704-12; A5547-127	*pat*	Bayer CropScience	1996 (U.S.)
Maize	T14, T25	*pat* (syn)	Bayer CropScience	1995 (U.S.)
	DLL25 (B16)	*bar*	Monsanto	1996 (U.S.)
Cotton	LLCotton25	*bar*	Bayer CropScience	2003 (U.S.)
Rice	LLRICE06, LLRICE62	*bar*	Bayer CropScience	2000 (U.S.)
	LLRICE601	*bar*	Bayer CropScience	2008 (U.S.)
Canola	HCN92 (Topas 19/2)	*bar*	Bayer CropScience	1995 (Canada)
	HCN28 (T45)	*pat* (syn)	Bayer CropScience	1996 (Canada)
	MS8, RF3 (male-sterile)	*bar*	AgrEvo	1996 (U.S.)
Sugar beet	T120-7	*pat*	Bayer CropScience	2001 (Canada)

Genes: *bar*: bialaphos resistance gene derived from *Streptomyces hygroscopicus* which eliminates phosphinothricin N-acetyltransferase activity by acetylation; *pat*: gene derived from *Streptomyces viridochromogenes* strain Tü 494 which eliminates phosphinothricin N-acetyltransferase activity by detoxifying L-phophinothricin through acetylation.

Table 3. Transgenic crop events/varieties developed for bromoxynil, dicamba, 2,4-D and imidizolinones

Herbicide	Crop	Event	Gene	Developer	First Approval (Yr)
Bromoxynil	Cotton	BXN	*bxn*	Calgene/Monsanto	1994
	Canola	Oxy-235	*bxn*	Bayer CropScience	1997 (Canada)
Dicamba	Soybean	MON87708	*aad-1*	Monsanto	2011
2,4-D	Maize	DAS40278	*aad-1*	Dow AgroSciences	2012 (Canada)
Imidazolinones	Soybean	CV127	*csr1-2*	BASF	2009 (Brazil)

Genes: *aad-1*: the synthetic form of this gene, *aryloxyalkanoate dioxygenase 1*, detoxifies 2,4-D by side-chain and also r-enantiomers of aryloxyphenoxypropionate herbicides; *bxn*: derived from *Klebsiella pneumoniae* subsp. *Ozaenae* which produces nitrilase enzyme. *csr1-2*: modified acetohydroxyacid synthase large subunit (AtAHASL) derived from *Arabidopsis thaliana*.

Table 4. Some of the transgenic stacked varieties/hybrids developed in different crops since 2008

Herbicide	Crop	Event	Gene	Developer	First Approval (Yr.)
Dicamba+glyphosate	Soybean	MON87708	*dmo+cp4 epsps* (*aroA*:*CP4*)	Monsanto	2015
Glyphosate+isoxaflutole	Soybean	FG72	*2mepsps + hppdPF W336*	Bayer CropScience	2013
Glyphosate+chlorsulfuron	Soybean	DP 356043	*gat4601+gm hra*	DuPont Pioneer	2008
Glufosinate+2,4-D	Soybean	DAS 68416-4	*pat+aad12*	Dow AgroSciences	2011
Glufosinate+mesotrione	Soybean	SYHT0H2	*pat+avhppd-03*	Syngenta & Bayer	2014
Glyphosate+glufosinate + 2.4-D	Soybean	DAS-44406-6	*2mepsps+pat+aad12*	Dow AgroSciences	2014
Glyphosate+ ALS Inhibitor	Maize	98140	*gat4621+gm-hra*	DuPont	2009
Glyphosate+ Glufosinate	Maize	GA21 x T25	*mepsps+pat* (syn)	Syngenta	2014 (S. Korea)
Glyphosate+2,4-D	Maize	DAS-40278-9 x NK603	*cp4 epsps* (*aroA*:*CP4*)*+aad-1*	Dow AgroSciences	2013 (Canada)
2,4-D+ACCase inhibitors+ glyphosate	Maize	DAS-40278-9 NK603	*aad-1+zm-hra+cp4 epsps* (*aroA*:*CP4*)	Dow AgroSciences	2014
Imidazolinones+glyphosate	Maize	--	--	DuPont Pioneer	2009
Glufosinate+dicamba	Cotton	MON 88701	*bar+dmo*	Monsanto	2013
2,4-D+glufosinate+glyphosate	Cotton	--	*aad-1+bar+cp4 epsps* (*aroA*:*CP4*)	Dow AgroSciences	---
Dicamba+glufosinate+glyphosate	Cotton	MON 88701 x MON 88913	*dmo+bar+ cp4 epsps* (*aroA*:*CP4*)	Dow AgroSciences	2015 (Japan)
Glufosinate+glyphosate+fertility restorer	Canola	MON88302 x RF3	bar+ *cp4 epsps* (*aroA*:*CP4*)*+barstar*	Monsanto	2014
Glufosinate+glyphosate+male sterile+fertility restorer	Canola	MON88302 x MS8 x RF3	bar+ *cp4 epsps* (*aroA*:*CP4*)*+barnase+ barstar*	Monsanto	2014

Genes: *aad-1*: aryloxyalkanoate dioxygenase 1 from *Sphingobium herbicidovorans*; *bar*: bialophos resistance gene from *Streptomyces hygroscopicus*; *barnase*: (a portmanteau of "**BA**cterial **R**ibo**N**ucle**ASE**) from *Bacillus amyloliquefaciens*; *barstar*: *barnase* inhibitor from *Bacillus amyloliquefaciens*: *dmo*: dicamba mono-oxygenase derived from *Stenotrophomonas maltophilia* strain D1-6; *gm hra*: (*Glycine max* herbicide-resistant acetolactate synthase) which encodes GM-HRA protein: *gat4621*: glyphosate *N*-acetyltransferase (*gat*) gene derived from *Bacillus licheniformis.*

Non-transgenic herbicide resistance

Herbicide resistant crops can be generated for some herbicides by selecting for target mutations in plant populations or tissue culture or by mutation breeding (Green and Castle 2010, Green and Owen 2011). This approach to produce HR crops has worked for herbicides (ALS and ACCase inhibitors) with relatively plastic molecular targets in which resistance evolves easily (Van Alfen 2014). This plasticity was proven by the fact that 160 and 48 species, respectively, have evolved resistance to ALS and ACCase herbicides that target these two enzymatic sites, almost exclusively due to target site mutations (Heap 2018).

There are different non-transgenic techniques to create crops with resistance to a number of ALS- and ACCase-inhibiting herbicides. These include, a) tissue culture selection, b) pollen mutagenesis, c) microsopore selection, d) seed mutagenesis and e) gene transfer from close weedy relatives that have evolved resistance. As the mutated genes only generate isoforms of enzymes that are already in the crop, and there is no insertion of new DNA into the genome, unintended consequences of the mutation are considered highly unlikely. Due to this, regulatory approval of the genetics of such crops is not needed. The first non-transgenic HR crop was developed in 1993 when a sulfonylurea-resistant soybean was commercialized. This was before the first transgenic HR soybean was commercialized.

Imidazolinone (IMI)-resistant crops have been the most successful non-transgenic HR crops. Evolution of weeds resistant to ALS-inhibiting herbicides occurs relatively quickly. Therefore, one might expect a problem with IMI-resistant weeds in these crops after only a few years.

IMI-resistant crops can be used to control parasitic weeds, in that imidazolinones translocate to metabolic sinks, thus affecting these weeds (e.g., *Striga* spp.). Seeds of resistant maize can be coated with IMI herbicide to provide *Striga* control (Kanampiu *et al.* 2009) and it can provide season-long control of this parasitic weed (Ransom *et al.* 2012).

Several non-transgenic events have been developed in soybean, maize, canola, rice, wheat, sunflower and sugarcane. Herbicides include the ALS-inhibiting imidazolinones (imazethapyr) and sulfonylureas and the ACCase-inhibiting cyclohexanediones (sethoxydim).

Non-transgenic crop events/varieties

BPS-CV127 is imidazolinone-resistant soybean event was developed by inserting the *csr1-2* gene derived from *Arabidopsis thaliana* to express AtAHASL (altered acetohydroxyacid synthase large unit) protein of 670 amino acids to confer resistance to IMI herbicides. This process involves transformation of embryonic axis tissue obtained from apical meristem of a soybean Brazilian cultivar 'Conquisita'. This line was first approved by Brazil in 2009, followed by several countries.

Non-transgenic IMI herbicide-tolerant maize lines have been developed by using selection-based and mutagenesis-based approaches. Using the former approach, maize embryonic cell cultures were subjected to sub-lethal doses of IMI herbicides and sectors of rapidly growing tissue are subsequently sub-cultured. These subcultures were then treated in successive selection cycles of increasing herbicide concentrations. The resistant cell lines were selected and plants regenerated in the presence of IMI herbicides. This method enabled development of two lines 'XA17' and 'XI12'. Selection-based approach was used by Pioneer Hi-Bred to develop 3417R maize line which was approved by Canada in 1994 (Health Canada 1999a).

In the mutagenesis-based approach, IMI-resistance is induced through chemical mutagenesis. In this, the pollen of a maize line is exposed to chemical mutagens followed by employing the mutagenized pollen to fertilize the parent line and screening the progeny for IMI herbicide tolerance. This method was used by Zeneca Seeds to develop the imazethapyr-resistant line EXP1910IT (Health Canada 1999b).

Regarding sethoxydim, two re-generable, friable, embryogenic callus cultures have been selected from a maize tissue culture of 'A188' x 'B73' cross in a medium containing this cyclehexanedione herbicide (Parker et al. 1990; Tan and Bowe 2012). These sethoxydim-tolerant callus culture lines, S1 and S2, exhibited 100- and >100-fold increases in sethoxydim resistance, respectively, compared to the unselected control callus lines. ACCase activity from S1 and S2 was inhibited 50% by sethoxydim concentrations that were 4-fold and 40-fold higher than concentrations required for 50% inhibition in wild type ACCase activity.

The sethoxydim resistance trait was introduced by BASF Canada into the registered maize hybrids 'DK412' and 'DK381' via tissue culture by a phenomenon known as somaclonal variation (Health Canada 1997; CERA 2001b). Somatic embryos of these maize hybrids were grown on sethoxydim-enriched culture media. The original sethoxydim tolerant mutant lines, which produced an altered ACCase enzyme while retaining its original catalytic properties, were selected from somaclonal variants from maize embryo tissue grown under sethoxydim selection pressure. From the somatic embryos that survived, the somaclonal variant cell line S2 was selected and subsequently regenerated. The regenerated plants were backcrossed at least six times with both parental lines of the hybrid DK412SR and DK404SR to transfer the sethoxydim-resistant trait. There was no new genetic material introduced into the genomes of these sethoxydim-tolerant lines as a result of the modification. Performance factors to measure the growth and development of DK412SR and DK404SR maize lines were comparable to the performance factors for unmodified maize lines and were within the normal ranges for the characteristics tested. These non-transgenic maize lines, primarily intended for animal feeding, were made available for commercial use in Canada in 1997 (Health Canada 1997).

Global adoption of biotech crops

Ever since the first commercialization of transgenic herbicide resistant crops on 1.73 million ha in 1996 in the U.S., beginning with the glyphosate-resistant (GR) maize, farmers around the world have readily adopted transgenic crops such as maize, soybean, cotton, rapeseed (canola), lucerne (alfalfa) and sugar beet. With an area of 189.8 million ha (469 million acres) under biotech crops in 2017 and 3% annual growth, global agriculture has witnessed about 110-fold growth during the past 22 yr. This makes biotech crops the fastest adopted crop technology in the history of modern agriculture.

Among the four major transgenic crops in 2017, soybean led with 94.1 million ha at 49.6% global biotech crop adoption. This was followed by maize (59.7 million ha; 31.4%), cotton (24.21 million ha; 12.8%) and canola (10.2 million ha: 5.4%). Other biotech crops accounted for just 1.29 million ha (0.8%).

The area under transgenic cultivation, doubled every 5 yr, now accounts for some 12% of global arable land. Biotech crops represented 35% of the global commercial seed market. Most of the commercially grown transgenic crops have one or both of two traits: herbicide (glyphosate) resistance and *Bt* (*Bacillus thuringiensis*) insect resistance. About 91% of the 18 million farmers who planted biotech crops in 29 countries in 2017 were risk-averse and resource-limited small farmers. The nine major countries which adopted biotech crops include USA (75 million ha), Brazil (50.2), Argentina (23.6), Canada (13.1), India (11.4), Paraguay (3.0), Pakistan (3.0), China (2.8) and South Africa (2.7) in that order which together account for 97% of biotech crops (ISAAA 2018). These nations excluding USA planted 109.8 million ha, accounting for over 58% of the global biotech crop area.

The herbicide-resistant (HR) transgenic (including the stacked herbicide-cum-insect-resistant ones) lines accounted for 80% of the global biotech area. Currently, two traits dominate the global biotech crops: herbicide resistance accounting for 65%, insect resistance 15% and a combination of the two (stacked) for 15%. Stacked-trait transgenics, whose use has been on the rise since 2000, are currently an important feature of biotech crops.

The HR maize event NK603 has the most number of global approvals. It is followed by herbicide- resistant soybean GTS 40-3-2, insect-resistant maize MON810, insect-resistant maize Bt11, insect resistant maize TC1507, herbicide-resistant maize GA21, insect-resistant maize MON89034, herbicide-resistant soybean A2704-12, insect-resistant maize MON88017, insect-resistant cotton MON531, herbicide-tolerant maize T25 and insect-resistant maize MIR162.

Biotech crops are considered to have made a positive contribution to global crop production and food security and improved the economic status of farmers who adopted them. However, they also accelerated changes in farming styles, affecting genetic diversity in agro-ecosystems of many countries that have adopted the biotechnology. For example, the adoption of HR biotech crops has changed traditional weed management practices and the biodiversity of crop and weed species. It also raised concerns about the biosafety to consumers besides the long-term profitability to farmers.

Benefits

The rapid adoption of HR biotech crops and their associated farm management practices suggest that they have become an important tool for managing weeds. These crops have changed weed management practices to a certain extent and made a significant contribution to the global production of crops, particularly maize, soybean, cotton and canola. Their adoption is generally attributed to low cost, simplified, more flexible and selective weed management options through the use of broad-spectrum, intrinsically non-selective herbicides (primarily glyphosate), a lower risk for crop injury and their compatibility with no-till or reduced-tillage systems. The benefits are of two kinds: pecuniary and non-pecuniary.

Pecuniary benefits

Pecuniary or direct benefits includes net farm income or profitability which is based on crop yields, market value of crop produce, production costs (seed and crop protection expenditure), and costs of fuel and labour. The most obvious pecuniary benefit is yield increase which is tangible and quantifiable.

HR crops have certainly increased the incomes of farmers who adopted them and countries which commercialized them. The incomes rose when biotech crops first became available in 1996 and they continued to rise even after 20 yr of their adoption. The cumulative global income benefit is also on the rise. Brookes and Barfoot (2018) reported that net economic benefits derived by four main GM-HR crops soybean, maize, cotton and canola at the farm level accounted to US$18.2 billion in 2016 and US$186.1 billion over the 21-yr period of 1996-2016. These benefits, derived by more than 16 million farmers, have been divided roughly 50% each to farmers in developed and developing countries. About 65% of these gains were due to yield and production gains while the remaining 35% coming from cost savings. GM soybean and maize have added 213 million tonnes and 405 million tonnes, respectively to the global production since their introduction in the mid-1990s. This gain is expected to increase over the years as area under biotech crops increase.

In 2017, the global market value of biotech crops was US$17.2 billion. It represented 30% of the US$56.02 billion global commercial seed market. The country-wise gains during the 1996-2017 period were in the order of US$80.3 billion in USA, US$23.7 billion in Argentina, US$21.1 billion in India, US$19.8

billion in Brazil, US$19.6 billion in China US$8 billion in Canada, with other countries accounting for US$13.6 billion. For 2016 alone, six countries gained the most economically from biotech crops. These were USA (US$7.3 billion), Brazil (US$3.8 billion), India (US$1.5 billion), Argentina (US$2.1 billion), China (US$1 billion), Canada (US$0.7 billion), and others (US1.8 billion) for a total of US$18.2 billion.

Adoption of biotech crops uplifted the economic situation of 16-17 million small farmers and their families totaling >65 million people around the world (Brookes and Barfoot 2018).

Non-pecuniary benefits

Non-pecuniary or indirect benefits include the intangible impacts influencing the adoption of transgenic crops. These include greater weed management flexibility, reduced crop toxicity, increased savings in time and equipment usage, improved quality of the crop produce, lesser impact on the environment, lower potential damage of soil-incorporated residual herbicides to rotation crops, etc. Some of these benefits are discussed in two categories: farm level and environmental level.

The primary impact of transgenic HR technology at the farm level is on providing a more cost-effective, easier and better weed control as against only a better weed control (regardless of cost) obtained from conventional method, even if crop yields remain the same in both technologies. In conventional cultivation, broad-spectrum, nonselective postemergence herbicides such as glyphosate, glufosinate, etc. are applied after the crop is established. When these are applied, the crop is very likely to be sensitive so as to suffer a setback in growth. This problem is eliminated when HR crop variety is used because the crop has already been engineered to be resistant to the herbicide.

HR technology allows for the ‘over the top’ spraying of biotech crops with broad-spectrum herbicides such as glyphosate, glufosinate, etc. that target both grass and broadleaf weeds but do not harm the crop itself.

HR crops and their associated farm management practices also enabled the control of several weed species congeneric to the crop. One example is weedy rice (*Oryza sativa* f. *spontanea*: red rice) (Gealy et al. 2009), considered as one of the most troublesome, difficult-to-manage and economically damaging weeds in cultivated rice (Ziska *et al.* 2015). Herbicide selectivity is generally based on the crop being able to metabolize and inactivate the herbicide more rapidly than the weed species. In the case of weedy and cultivated rice, no such difference exists due to their genetic similarity. With the introduction of imazamox-tolerant non-transgenic conventionally-bred herbicide resistant (CHR) rice varieties, effective control of weedy rice became possible (Ziska *et al.* 2015). Similar problems occur with sexually-compatible weeds in other crops such as oilseed rape (canola) and sunflower (Muller *et al.* 2009).

Another indirect farm level impact of HR technology is to provide more cost-effective and better weed control. The main source of additional production is the facility to adopt conservation production systems (no-till and reduced-till) and shorten the production cycle, thus enabling taking second crop in a relatively weed-free situation. Growing another crop following a HR crop would certainly raise farm income. Besides, conservation system eliminates or reduces pre-planting soil cultivation or seedbed preparation to eliminate weed growth. As a result, tractor fuel use for tillage is reduced, soil quality is possibly enhanced and soil erosion lowered. Conservation systems also contribute to reducing soil erosion and moisture loss, fossil fuel use carbon dioxide emissions, nitrogen and pesticide leaching and improving soil structure (Cerdeira and Duke 2010, Basso *et al.* 2011, Carpenter 2011). This provides for additional monetary savings in the form of lower labour and fuel costs associated with plowing, besides aiding in additional soil moisture retention and reduced soil erosion (Brookes and Barfoot 2012).

Improved weed control may contribute to reduced harvesting time and enhanced quality of the harvested crop. Higher quality of crop produce may fetch higher market prices. Adoption of HR crop avoids the potential damage caused by soil-incorporated residual herbicides to follow-on (rotational) crops while reducing the need to apply herbicides to them because of earlier improved levels of weed control.

Another non-pecuniary benefit is the impact of HR biotech crops on the environment. Their adoption saved 671 million kg (ai) of herbicides and insecticides during 1996-2016, with a gross saving of 8.2%. In 2016 alone, these crops saved 48.5 million kg pesticides, a saving of 8.1%. About 70% these savings were attributed to herbicides. These gross savings reduced impact on environment (Environmental Impact Quotient: EIQ) to the tune of 18.3% over the 21 yr period, with 18% being in 2016 alone.

Increases in atmospheric levels of greenhouse gases such as carbon dioxide, methane and nitrous oxide are detrimental to the global environment. Therefore, if the adoption of HR crop technology contributes to a reduction in the level of greenhouse gas emissions from agriculture, this represents a positive development for the world. Brookes and Barfoot (2018) reported that biotech crops reduced CO_2 emissions in 2016 by 27.1 million kg, equivalent to taking 16.7 million cars off the road during the year. The largest fuel-related reductions in CO_2 emissions have come from where HR soybean varieties have been adopted.

Based on savings arising from the rapid adoption of no till/reduced tillage farming systems in North America and South America in 2011, an extra 5,751 million kg of soil carbon was estimated to have been sequestered. This was equivalent to 21,107 million tonnes of CO_2 that has not been released into the global atmosphere. The cumulative savings over a longer period of growing transgenic HR crops would certainly be much higher. The reduction in GHG emissions and its quantification are dependent on several variables like crop type, crop duration, cropping system, soil type and environmental conditions, etc. Thus, transgenic HR crops have the potential to reduce emissions of greenhouse gasses in substantial quantities.

Among the GM-HR crops, maize reduced herbicide use by 193.1 million kg, a 10.1% reduction, and this led to a concomitant reduction (12.5%) in the impact on the environment. Regarding transgenic soybean, with largest area under it, herbicide use came down by 12.5 million kg and it translated into a 15.5% decrease in impact on the environment. Of the environmental benefits derived by using HR crops, developed countries have been the major beneficiaries (55%) than developing nations (45%). This situation may very soon turn in favour of developing countries as they bring in larger area under transgenic crops.

Biotech crops have the potential to lower greenhouse gas (GHG) emissions by saving on the fuel by reducing the number of herbicide applications. In the case of HR crops, particularly those engineered for resistance to glyphosate and glufosinate, adopting conservation (reduced or no-tillage) farming systems would lead to savings in CO_2 emissions. Brookes and Barfoot (2013) estimated that the reduction of CO_2 emissions consequent to growing biotech crops in 2011 was to the tune of 1,886 million kg and this lowered the fuel usage by 706 million L. The cumulative reduction in gas emission over the period of 1996-2011 was 14,610 million kg arising from a saving of 5,472 million L of fuel.

Import of herbicide-resistant biotech crops to India

India has adopted biotech crops in 2002, but this was restricted to insect-resistant (IR) *Bt* (*Bacillus thuringiensis*) cotton as it introduced Monsanto's lepidopteran-insect tolerant Event MON531 (Bollgard I) and its three modified hybrids (Mech-12 *Bt*, Mech-162 *Bt*, and Mech-184 *Bt*} developed by Monsanto and its partner Maharashtra Hybrid Seed Company (Mahyco). These hybrids, which control bollworm, were developed by crossing Monsanto's Event MON531 (Bollgard) with local elite Indian varieties. In 2006, Monsanto commercialized another variety, Event MON15985 (Bollgard II), which carried two IR genes, *cry1Ac* and *cry2Ab2*.

Consequently, the country achieved a great stride in cotton production with a quarter of market share in global cotton production in 2017 when it harvested 6.21 million tonnes, the most by any nation. Beginning with an area of 50,000 ha in 2002, the biotech cotton area increased 6% from 10.8 million ha in 2016 to 11.4 million ha in 2017, equivalent to 93% of gross cotton area of 12.24 million ha. This IR (*Bt*) technology boosted cotton yields to 500 kg ha^{-1}. India gained economic boost to the tune of US$21.1 billion ([1] 2110 crore) during the 1996-2017 period. The benefit in 2017 alone was US$ 1.5 billion ([1] 150). The country now aims to reach the next level of yield target of 700 kg ha^{-1}. However, this can only be achieved with the introduction of new generation biotech traits including stacked traits, smart agronomy and high yielding cotton cultivars. *Bt* cotton varieties are considered to have helped minimize the damages caused by bollworm, reduce insecticide use and enhance net income of farmers.

Currently, there is no herbicide resistant biotech crop that is permitted by the national government to grow. The glyphosate-resistant cotton has not received the approval Genetic Engineering Approval Committee (GEAC) of Government of India.

Glyphosate, commercialized in 1974 by Monsanto, was approved in India for perennial weed management in mature tea in 1981 after a series of field tests for six years at Tocklai Tea Research Institute, Jorhat, Assam. Later, its use has been extended to a few perennial crops only when used as directed spray. Currently, there are many players involved in making glyphosate available.

Glyphosate is not recommended for use in cotton. No license of approval has been granted by Government of India for growing HR cotton varieties. However, farmers have been using glyphosate in insect-tolerant cotton (Bollgard III) fields. Besides, some seed companies have been producing and selling glyphosate-resistant 'Roundup Ready Flex' (MON88913: Table 1) cotton seeds illegally for unauthorized use by farmers in key cotton growing states across India. Farmers are swayed by the multiple benefits that genetically modified varieties offer. Currently, around one million kg of glyphosate are being sold, with much of this quantity being used in IR cotton crop in several states.

In a bid to curb the illegal use of glyphosate in insect-tolerant cotton, state governments have suggested restrictive use of glyphosate in agricultural and horticultural crops in general and especially in cotton in order to stop the spread of illegal HR cotton.

Future of HR biotech crops

Herbicide resistant biotech crops have the potential to adopt effective weed management practices. In many a case, they will also lead to cost-effective weed management both in the short-term and medium-term. However, in the long-term their impact needs to be considered very carefully in the light of limitations and concerns discussed below.

Limitations and concerns

Commercial production of biotech crops has aroused serious concerns about their biosafety. Biosafety issues have become a crucial limitation to their further development. Genetically engineered crops are a heterogeneous group. As such, it is not reasonable to lump all of them together. Therefore, it would be prudent to assess the biosafety of each of the transgenic crops separately.

Development of transgenic crops is seen more a profit-driven rather than need-driven process. Therefore, the thrust of the genetic engineering industry is not really viewed to solve agricultural problems, but to create profitability (Altieri 1998). Although several universities and research institutions are also simultaneously involved in this field, their research agenda is being increasingly influenced by private sector in ways never seen in the past. The challenge for these organizations is how to ensure that ecologically sound aspects of biotechnology are researched and developed while carefully monitoring and controlling the provision of applied non-proprietary knowledge to the private sector, farmers and consumers while making such knowledge available in the public domain for the benefit of society (Altieri 1998).

Currently, there is a great deal of confusion on the concerns, both real and perceived, attributed to biotech crops. These concerns are related to agro-ecology, evolution of resistant weeds, food safety and soil ecosystem.

Agro-ecological concerns

These are related to gene flow from biotech crops to, a) conventional crop varieties, b) landraces and wild/weedy relatives and c) to unrelated organisms.

When genes move from biotech crops to their non-biotech counterparts through seed-, vegetative organ-, or pollen-mediated gene flow, it could lead to 'adventitious mixing' of varieties of both crops. This 'gene-pollution' often occurs where both are planted in close proximity. The frequencies of gene movement mediated by pollen depend essentially on the breeding systems and quantity of pollen of crops (Lu 2008). A significant gene flow to non-biotech crops may subsequently move to weedy and wild relative populations.

The pollen-mediated gene flow is dependent on crop. For soybean, cross-pollination is not a problem, but considerable outcrossing can occur with maize, rice, sugar beet and canola. Wheat and rice are predominantly self-pollinating, but cross-pollination does occur at a low range.

Evolution of herbicide resistant weeds

In reality, crops do not select for HR weeds, but herbicides do. Therefore, development of HR weeds is not due to a biotech crop, but it is a due to the herbicide used. When an herbicide is used continuously over a time period, evolution of resistance is a natural phenomenon, regardless of crop culture.

Currently, glyphosate resistant (GR) transgenic crops account for about 90% of HR biotech crops. Resistance of weeds to glyphosate began in 1996 when the monocot *Lolium rigidum* Gaudin was found resistant in Victoria, Australia. This was 22 yr after glyphosate became commercially available. This was also about the time GR transgenic crops (soybean, maize, cotton and canola) were being adopted. Since then, 41 more species (22 dicots and 19 monocots) became resistant to this non-selective herbicide (Heap 2018). Around 30 of them were from

GR transgenic crops in countries which adopted them, particularly USA, Brazil, Argentina, Canada and Colombia. The major weed species include *Amaranthus palmeri* S. Watson, *Amaranthus tuberculatus* (Moq.) Sauer (= *A. rudis* Sauer), *Ambrosia artemisiifolia*,L, *Ambrosia trifida*L., *Eleusine indica* L. (Gaertn.), *Kochia scoparia* (L.) Schard. *Poa annua* L., *Sorghum halepense* (L.) Pers. and various *Conyza* and *Lolium* species. The over-reliance on glyphosate to control weeds contributed to the evolution of multiple-resistant weed populations.

As more global acreage is treated continuously with other herbicides like ALS, ACCase and PS II inhibitors, in both cropping systems, faster and greater emergence of weed species resistant to them may become a serious problem in future. This will invariably cause weed shifts, thus requiring newer weed management strategies to combat the problem.

Food safety

The widespread consumer concern about transgenic crops is the potential risks they have on human and animal health. These risks associated with consumption of the edible parts of crops and foods derived from them. The issues surrounding foods and feeds of HR biotech crops are broadly grouped into: a) nutrient levels, b) allergenicity, c) horizontal transfer and antibiotic resistance, d) consumption of foreign DNA and e) the promoter such as CaMV used during genetic modification.

Nutrient Levels. A major concern about transgenic crops is whether the transgene will alter nutrient levels of foods and feeds derived from them. Transgene integration and/or transformation and tissue culture during transgenic process may induce unintended genomic alterations such as deletions, insertions and rearrangements, which may generate secondary or pleiotropic effects in transgenic plants (Cellini *et al.* 2004, Garcia-Canas *et al.* 2011, Herman and Price 2013).

Allergenicity. The possibility of allergic reactions to food as a result of genetic engineering is a powerful emotional issue because exposure of individuals to biologically active genes from non-plant sources can have major effects on their gastrointestinal tract. Even people who have never experienced an allergic reaction may worry that they are being exposed to new substances for which there is little track record of safety or harm. It is also likely that in addition to the effects on the gastrointestinal tract, the size, structure and function of the internal organs will be affected, particularly in young and rapidly growing humans and animals.

Horizontal Gene Transfer and Antibiotic Gene Resistance. Horizontal gene transfer (HGT) refers to the transfer of genetic material between organisms as in the case of plants and microorganisms, unlike the parent-to-offspring channel in vertical transfer. The main concern of HGT is the possibility of transfer of transgenes to humans from plants used directly as food (also processed food) or indirectly as feed to animals used for food. Transfer occurs by the passage of donor genetic material across cellular boundaries, followed by heritable incorporation to the genome of the recipient organism. HGT plays an important role in the evolution of bacteria that can degrade novel compounds such as insecticides, herbicides, *etc*.

Consumption of Foreign DNA. When a food derived from a transgenic crop is consumed, we eat the DNA of bacteria and viruses without knowing that we do so. Some of this DNA is similar to human DNA, but much of it is foreign to us. Most of the ingested DNA is broken down into more basic molecules during digestion process, while a small amount is not. This may either be absorbed into the blood stream or excreted in the feces. In fact, DNA can persist in the gastrointestinal tract and become available for uptake by intestinal bacteria. Although the colon is the preferential site for transformation of these bacteria, the amount of DNA reaching it may only be a fraction of what is consumed.

CaMV Promoter. The cauliflower mosaic virus 35S is used as a preferred promoter in transgenic crops. It is used to “turn on” the gene inserted in the host genome. It causes CaMV disease in cauliflower, broccoli, cabbage and rapeseed. It can be horizontally transferred and cause disease, carcinogenesis, mutagenesis, reactivation of dormant viruses and generation of new genes (Hodgson 2000, Artemis and Arvanitoyannis 2009). However, normal foods containing CaMV is not highly-infectious and cannot be absorbed by mammals (Ho et al. 2000). In fact, humans have been ingesting CaMV and its 35S promoter at high levels, but have never been reported to cause disease or recombine with human viruses (Paparini and Romano-Spica 2004).

Soil ecosystem

Soil ecosystem, 80% of which is accounted by soil-borne communities dominated by microbes, is one of the least understood areas in the risk assessment of biotech crops. Rhizosphere microbes play a major role in nutrient mobilization, and cycling and decomposition of wastes. Any impact that biotech plants have on the dynamics of the rhizosphere and root-interior microbial community may cause either positive or negative effects on plant growth and health and, in turn, ecosystem sustainability. Soil microbial communities have several opportunities to interact with novel plant gene products during crop growth. After harvest, decomposition of plant litter and straw can release novel proteins into the soil environment.

A manifold increase in glyphosate application in GR biotech crops since 1996 has been reported to have several adverse effects, including immobilization of nutrients, increase in plant diseases due to weakened plant defenses and enhancement of pathogen virulence. These changes have apparently been caused by root exudates released by GR crops following glyphosate application (Bromilow *et al.* 1993). Thus, considerable concern exists regarding the potential detrimental effects of rhizosphere microbes on GR transgenic crop productivity resulting from either direct effects of glyphosate or its indirect effects on plant physiological functions (Zobiole *et al.* 2011).

Application of glyphosate results in reduced root nodulation in GR soybean crop, while delaying nitrogen fixation and plant biomass accumulation (Zablotowicz and Reddy 2004). However, the severity of these effects was dependent upon formulation and number of applications of glyphosate aside from GR cultivar. Powell et al. (2009) reported significant differences in nodulation among six GR and three near-isoline GR cultivars, but these were not related to glyphosate resistance.

Glyphosate application in GR soybean field may cause reduced nutrition uptake, leading to enhanced occurrence of many diseases which, in turn, detrimentally impact many beneficial soil microbes. The EPSPS enzyme present in GR soybean is considerably less efficient than wild-type enzyme, producing insufficient amounts of phytoalexins (key defense components associated with shikimate pathway) to prevent fungal infection (Gressel 2002). Besides, EPSPS also lowers the shikimate-dependent lignification of cell walls at or around the infection site. Decreased lignin content may also be due to the reduced photosynthesis in soybean caused by glyphosate (Zobiole *et al.* 2010).

Although glyphosate is rapidly inactivated by soil adsorption, it may serve as a substrate for some microorganisms. Kremer and Means (2009) found higher colonization of roots by *Fusarium* spp. when field-grown GR transgenic soybean cultivars were applied with glyphosate over a 10-yr period (1997-2007), while plants receiving no or conventional postemergence herbicides exhibited low *Fusarium* colonization. The non-transgenic cultivars had the lowest root colonization by *Fusarium*. This colonization increased as soybean growth progressed and glyphosate rate increased (Zobiole *et al.* 2011). Reduced production of both lignin and phytoalexin allows increased root colonization by *Fusarium* in plants injured by glyphosate (Johal and Rahe 1988).

Socio-economic consequences

Ensuring coexistence of biotech and conventional crops and products derived from them will inevitably entail additional costs in several ways. The costs include those required to, a) enforce coexistence measures imposed by regulators, both during and after cultivation, b) for testing of crop produce and products, c) for identifying and quantifying the content of transgenic material in non-transgenic material and d) for compliance of labelling and traceability requirements. Additionally, farmers may suffer income losses due to restrictions in crop choice and management. Neighbouring farmers could impose restrictions if a farmer decides to grow a transgenic crop. Besides, spatial restrictions, temporal cultivation may occur due to irreversibility. In a field where transgenic crop is raised, it could temporarily be difficult to meet the 0.9% tolerance threshold if a farmer decides to go back to a non-transgenic cropping system. In this process, a conversion time might be required to deplete transgenic seeds from the seedbank and/or control of volunteers and weedy/wild relatives that may contain the transgene.

Coexistence of biotech and non-biotech crops in the same region also has social consequences. Farmers who decide to grow transgenic crops need to, a) seek approval of neighbouring farmers, b) notify their crop details and seek permission from government regulators, c) consider ethical issues that may arise in connection with the use genes from non-plant sources, d) study the positive and negative effects of biotech crops in relation to sustainable development, e) assess the risks of the extinction of traditional varieties, f) weigh corporate control of seed and g) bear in mind the legal liability of biotech crop cultivation.

Coexistence of biotech and conventional crops

Adventitious mixing and preventive measures

As agriculture is an open system, certain amount of adventitious mixing is unavoidable. The on-farm sources of such mixing between biotech and conventional crops include seed impurities, pollen flow between neighbouring fields, volunteer plants originating from seeds or vegetative plant parts from previous biotech crops and seeds left behind inside the equipment used for various operations.

The existing measures to ensure seed purity in conventional crop production may also be applied within the context of limiting the adventitious content of transgenic material in seeds and plant products. These include: a) the use of certified seed, b) spatially isolating fields of the same crop, c) erecting pollen barriers around fields, d) scheduling different sowing and flowering periods, wherever possible, e) limiting carryover of transgenic volunteers into the following crop through the extension of cropping intervals, f) cleaning agricultural machinery and transport vehicles for seed remnants, g) controlling volunteers and wild/weedy relatives, h) applying effective post-harvest tillage operations, i) retaining records of field history and j) the voluntary clustering of fields. The drastic preventive coexistence measure is probably banning the cultivation of transgenic crops in a certain region.

Development of illegal HR biotech crops in India is seen more a profit-driven rather than need-driven process. Therefore, the thrust of the genetic engineering industry is not viewed to solve agricultural problems, but to create profitability.

The amount farmers pay for use of the technology varies by country. Pricing of technology (all forms of seed and crop protection technology including HR technology) depends on the level of benefit that farmers are likely to derive from it. In addition, it is influenced by intellectual property rights (patent protection, plant breeders' rights and rules relating to use of farm-saved seed). In countries (e.g. India) where governmental regulations on price control are weak, biotech crop seed suppliers may tend to price their seed at abnormally higher rates. The concerned countries need to have strict price control structures in place for biotech crops seeds.

Coexistence of biotech and conventional food products

Labelling, a prerequisite for coexistence of transgenic and non-transgenic foods, is an important issue related to biotechnology. There is no federal or state law in the U.S. that requires food producers to identify whether foods were produced using genetic engineering. Despite such heavy consumption of transgenic foods by American consumers, the US Food and Drug Administration does not require safety studies of such foods. Considering that transgenes have been derived from bacteria and viruses, 9 out 10 people want these foods labelled (Bartolotto 2013). The biotech companies, however, do not.

Consumers in many parts of the world are now demanding labelling so they can exercise choice between foods that have originated from biotech, conventional, or organic crops. This requires a labelling and traceability system as well as the reliable separation of transgenic and non-transgenic foods at production level and throughout the whole processing chain.

Since recently, several food products derived from biotech crops grown outside of India have been flooding the super markets in the country. These imported "fancy" products such as pan-cake syrups, multigrain cereals, corn puffs, oils from canola and cotton, silken tofu, etc. Some of the imported infant food products have their origin in biotech crops. These packages do not carry GM labels. Besides, local manufacturers are supplying the oil from seeds derived from biotech cotton. The Food Safety and Standards Act, 2006 (Section 22) does not allow manufacture, import or selling of GM food products in India unless approved by it.

Basically, consumers have the right to know what is in the foods they consume. It will be a travesty of justice to deny it. It may not be too long before consumers' demands are met by global governments.

Management of herbicide-resistant crops

Depending on the specific herbicide regime, the adoption of HR transgenic crops can pose several environmental and socio-economic challenges, one of which is to exacerbate evolution of HR in weeds. The use of a single herbicide for a longer period changes the weed flora, and increases the selection of HR weed biotypes. Diversification in crop systems and weed management tactics reduces the risk of weeds evolving herbicide resistance(s) and promotes biodiversity.

Therefore, the most effective and sustainable use of HR crops would be to make it a component of an integrated weed management (IWM) approach. IWM prescribes the use of multiple tactics, both chemical and non-chemical, to suppress weed populations, and to prevent or delay HR evolution. The potential benefits of IWM with HR crops are seldom realized because a wide range of technical and socio-economic factors hamper the transition to IWM (Lamichhane *et al.* 2016).

Therefore, several tactics may be used to integrate HR crops within the framework of IWM. These include a) herbicide-based practices, b) rotation of crops, c) cover crops and intercropping, d) tillage, e) using competitive crop genotypes, f) biological management and g) manual and mechanical weeding.

Herbicide-based practices

The herbicide-based practices should take into account factors affecting evolution of weed resistance such as frequency, number, dominance and fitness of genes conferring resistance to an herbicide. Herbicide rotations and mixtures can delay evolution of HR evolution. Rotation of effective herbicides with different action sites is the most widely implemented HR management strategy. This can delay the evolution of HR (except for non-target site resistance which may continue to evolve under this strategy) (Beckie and Reboud 2009). A better tactic would be the use of herbicide mixtures, and this is considered more effective than rotating herbicides with different modes of action (Beckie and Reboud 2009, Evans *et al.* 2016). However, neither tactic is likely to prevent evolution of HR in weeds in the long run, and therefore is not a permanent solution. Herbicidal mixtures may delay evolution of resistance, but they do not prevent it. Applying reduced rates may support a more efficient use of herbicides. Although this is not a viable practice, it may be effective on more susceptible weed species. Weed species differ in their susceptibility to herbicides, and a low rate of one herbicide may be more effective than a full rate of another herbicide. Similarly, a low rate applied under optimal conditions may be more effective than a full rate applied at sub-optimal conditions (Kudsk 2014). However, sub-lethal herbicide rates can select for non-target site resistance, which is quantitatively inherited through accumulation of minor genes (Neve *et al.* 2014). They also increase the risk for cross-resistant evolution. As HR crops, in most cases, are tolerant to highly effective and broad-spectrum herbicides, it is likely that their adoption will promote the use of reduced rates of ALS inhibitors in imidazolinone-tolerant crops.

Crop rotation

Crop rotation can favour a more diverse composition of weed communities. It allows alternative weed control strategies to be used, and enables alteration of patterns and timings of soil disturbance, light transmission through the crop canopy and natural enemies infesting the crop, thereby diversifying the selection pressures on weed populations and making it ecologically more difficult for one weed species to dominate a weed community (Lamichhane *et al.* 2016). Diversity in crop systems (which include both the crops grown in rotation and the associated farm management practices) represents the best practice to mitigate risks related to herbicide resistance.

Despite obvious benefits, diverse crop rotations are difficult to implement. The benefits may only become apparent in the long-term. Moreover, the adoption of crop rotation will inevitably be hampered by market-driven production strategies. Major limiting factors in adoption of rotational crops include, a) the lack of markets available for a new crop introduced in the rotation, b) low economic returns, c) lack of suitable herbicide options for all rotational crops in the crop rotation and d) the necessity to implement weed management systems that are in tune with other pest management measures.

HR biotech crops may provide more effective herbicide solutions than currently available, enabling them to control a broader spectrum of weeds. Thus, it can be envisaged that access to HR crops and their associated farm management practices will incite some farmers to neglect crop rotation as a weed management measure, as this may no longer be a prerequisite to achieve effective weed control. In addition, re-cropping restrictions due to herbicide residue in soil may limit cropping options in the following year.

Cover crops and intercropping

Cover crops compete with weeds for space, light, water and nutrients aside from providing a suitable habitat for organisms that feed on weeds. Besides, cover crop residues that remain on the soil surface as mulches suppress weeds by reducing light transmittance, soil temperature and by releasing allelochemicals. However, the adoption of cover crops poses some challenges when, a) labour and time are limited and b) additional costs incurred with the purchase of seeds are involved. Besides, they cause reduction in soil moisture, possible build-up of insects and diseases in soil, difficulty in soil incorporation of herbicides and delay in crop seed germination.

Significant benefits can be obtained in terms of weed control when a proper combination of crop species is grown together for spatial diversification (Bilalis *et al.* 2010). Intercropping offers weed control advantages over sole crops, a) by suppressing weed growth through competition and allelopathy and thus more effectively use available resources at the expense of weeds and b) by providing yield advantages either using resources that are not exploitable by weeds or using converting resources to harvestable material more efficiently than sole crops.

Despite the advantages of intercropping offers, growing two or more crops simultaneously on the same field leads to more complex crop management and possible additional costs that may restrict their use by farmers. In the case of HR biotech crops, applying two different weed management systems on a single field may not be practical, because the chosen crops should be tolerant to the same herbicidal active substance. If crop choices or timing differences in crop life cycles are not managed properly, then these two crops can compete for water and nutrient resources, which may have negative effects on crop yield. The complexity of intercropping can make a given cropping system more vulnerable to environmental stresses.

Tillage

When tillage is used in conjunction with other cultural tactics such as cover crops and crop rotations, it can markedly reduce densities of weed population. Overall, weed population density and herbicide use tend to be lower under conventional tillage compared to reduced tillage systems, especially for perennial weeds that are markedly decreased under conventional tillage systems. In-crop tillage has more potential to directly replace some of the postemergence herbicides used, though tolerance to in-crop tillage varies by crop type and growth stage (Nazarko *et al.* 2005).

Fuel use, erosion, greenhouse gas emissions and loss of water from soils are greater in conventional tillage. Reduced tillage or ‘no-till’ system, generally associated with HR biotech crops, can also become a part of IWM. Weed seeds left on the soil surface have a higher mortality rate, partly due to predation. Moreover, crop residues left on the soil surface can further suppress weed growth.

Competitive crop genotypes

Cultivation of competitive crop genotypes, characterized by rapid germination and emergence, vigorous seedling growth, rapid leaf expansion, rapid canopy development and extensive root systems, is a potentially attractive option for IWM, because their use does not incur additional costs. For example, crop genotypes with high competitive potential have been identified in certain crops. The use of competitive plant genotypes alone can result in a 50% reduction in recommended levels of herbicides in wheat (Travlos 2012). The adoption of HR biotech crops may reduce the focus on crop competiveness because of the availability of effective herbicidal active substances for weed control such as glyphosate. Therefore, biotechnologists need to focus on using competitive crop genotypes with greater yield potential in developing HR biotech crops.

Biological control

Biological control aims to suppress weed populations below levels that cause economic injury instead of controlling them. While there have been a number of successful biological control programmes, biocontrol of weeds presents a range of challenges. These include economic feasibility, effectiveness of control agents, statutory and regulatory constraints for product registration, technological constraints in developing bioherbicides, environmental constraints and difficulties in utilizing pathogens and herbivores as biocontrol agents. The potential impact of HR biotech crops on biocontrol agents could be negative. Therefore, the interest in bio-agents for perennial weeds would likely be reduced for biotech HR crops. However, sub-lethal doses of glyphosate can work in synergy with microbial bio-agents as the former temporarily stops the growth of the weed, allowing time for the latter to establish and inhibit growth (Boyette *et al.* 2008, 2015).

Mechanical weed management

Depending on soil characteristics and conditions, mechanical weeding has proven effective on a range of crops. The inclusion of innovative technologies, including advanced sensing and robotics, in combination with new crop systems, might lead to a breakthrough in physical weed control in row crops resulting in significant reductions, or even elimination, of the need for hand-weeding. Inter-row cultivation and band spraying with an effective herbicide in a biotech crop could potentially reduce the risk of HR weeds to evolve. However, mechanical weeding requires greater fuel use, is more timeconsuming, and may result in more soil erosion, greenhouse gas emissions, loss of water from soils, and cause adverse effects on the flora and fauna if not applied correctly (Navntoft *et al.* 2007).

The adoption of biotech crops may lead to reduced interest in mechanical weeding. First, farmers consider them to be more cost-effective than mechanical weeding and they delay evolution of HR weeds. Second, they tend to promote conservation tillage systems that are less conducive to mechanical weeding.

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