Genetic Engineering of Crops for Insect Resistance

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Article Outline

Glossary

Coding sequence The part of a gene which determines the sequence of the protein product

Domain A region of a protein which forms a distinct 3-D structure, and will often form this structure even when separated from the rest of the protein

Genetic engineering Introduction of a specific DNA sequence into an organism by artificial means

Insect orders Lepidoptera=butterflies and moths; diptera=flies; coleoptera=beetles; hemiptera/homoptera=sucking insects such as aphids

Mutagenesis Alteration to a DNA sequence, often resulting in alteration to the sequence of a protein which the DNA specifies

Oligomerization Formation of polymers containing a relatively low number of repeating units

Proteolysis Introduction of breaks in the chain of amino acids making up a protein by a proteinase

Transgenic Organism into which a gene has been introduced by genetic engineering technology

Definition of the Subject

Genetic engineering of crops for insect resistance is the introduction of specific DNA sequences into crop plants to enhance their resistance to insect pests. The DNA sequences used usually encode proteins with insecticidal activity, so that in plants which contain introduced DNA, an insecticidal protein is present. However, other strategies to improve plant defenses against insects have been explored. Genetically engineered crops that are protected against major insect pests by production of insecticidal proteins from a soil bacterium, *Bacillus thuringiensis*, have become widely used in global agriculture since their introduction in 1996.

Introduction

Twenty years have elapsed since the first publications describing transgenic plants, which showed enhanced resistance to insect herbivores, as a result of the expression of a foreign gene encoding *Bacillus thuringiensis* (*Bt*) toxin [1–3]. In the intervening years, crops expressing these toxins have become widely used in global agriculture, and have led to reductions in pesticide usage and lower production costs [4] At the same time, the predictions made by lobby groups supporting "organic" crop production, that irreversible environmental damage would be caused by genetically engineered (GE) crops resistant to insect pests, have not been realized [5]. Despite all the controversy that GE crops have caused in many countries, it is difficult to dispute that the use of this technology to combat insect pests has had a positive impact on global agriculture.

This entry has two aims: first, to provide a summary of how and why *Bt* toxins have become the insect resistance genes of choice for commercial GE crop applications, and to anticipate some further developments of this technology; second, to consider some of the other approaches to engineering insect resistance in

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plants, and to assess their potential for future development in the development of sustainable agriculture.

Insecticidal Proteins from *Bacillus thuringiensis*

The presence of insecticidal toxins in the soil bacterium *Bacillus thuringiensis* (*Bt*) has enabled both the bacteria themselves, and genes derived from them, to be exploited as plant protectants. The toxicity is almost invariably based on proteins produced during sporulation of the bacteria, which form crystalline deposits associated with the spores. The insecticidal *Bt* proteins are encoded by genes present on plasmids, and the presence of these plasmids is the main feature which distinguishes *Bt* from other spore-forming bacilli [6]. Preparations of *Bt* spores have been used since the 1920s as a conventional, spray insecticide (and, as a "natural" product, are approved for use in organic agriculture), but their efficacy in the field is limited by inactivation and low persistence.

The ecological niche occupied by *Bt* appears to be simple to define. The life cycle starts with a spore and associated crystalline protein body which may be present in the soil. On being eaten by an insect, the protein deposit associated with the spore is dissolved and digested, converting the crystalline protoxin to an active toxin. The insect is then killed, and the carcass provides nutrients for the growing bacteria, which multiply rapidly. When the insect carcass is exhausted, the bacteria sporulate; the spores are dispersed, and the cycle recommences. However, this cycle is clearly too simplistic, as the target insects for *Bt* toxins are only rarely soil dwellers, and the dose of spores required to kill an insect larva is too large for dispersed spores to have much effect. Although *Bt* is widely distributed, levels of the bacterium in soils are generally too low to have any effect on insects, and spraying plants with spores does not result in persistent protection as a result of the establishment of a high bacterial population. The species has been described as an opportunistic pathogen, which has evolved the sporulation mechanism as a "backup" system to ensure its survival under unfavorable conditions [7]. *Bt* is naturally present in the phylloplane, as well as in soil, and has been detected on cabbage foliage [8], and in vegetative form on clover [9] at low levels, without any insecticidal effect. However, the insecticidal characteristic must be of benefit to the bacterium, since most of the insecticidal proteins are encoded by plasmids, and the plasmids are maintained in the *Bt* population as a whole, despite the obvious metabolic costs of producing large quantities of spore-associated proteins. Not only are toxin-encoding plasmids maintained, but there is also a huge reservoir of diversity in the toxins themselves, and much effort has been put into screening bacterial isolates for strains of *Bt* with novel pesticidal activities [10].

Bt toxins are now classified on the basis of amino acid sequence similarity (an earlier classification system based on pesticidal activity has been superseded), in a systematic hierarchical system [11]. For the purposes of this contribution, only the major distinctions need be considered. There are four types of insecticidal proteins produced by *Bt*:

1. Proteins associated with *Bt* spores, usually as crystalline deposits; three domain structure; single toxins; designated by the symbol Cry
2. Proteins associated with *Bt* spores, usually as crystalline deposits; binary toxins and other similar proteins, including truncated versions of three-domain toxins; also designated by the symbol Cry
3. Proteins associated with *Bt* spores, usually as crystalline deposits; single domain structure; cytolytic; single toxins; designated by the symbol Cyt
4. Proteins expressed vegetatively by *Bt*; single chain and binary toxins; designated by the symbol Vip

Each type of toxin is subdivided (on the basis of sequence similarity) into families (number; same number $\geq$45% sequence identity) and then further subdivided using capital letters (same letter $\geq$78% sequence identity), small letters (same letter $\geq$95% sequence identity) and numbers successively. The resulting system yields designations for specific toxins such as Cry1Aa. A single *Bt* strain can produce spores which contain only a single toxin, or a complex mixture, such as the *Bt* subspecies *israelensis*, whose spores contain Cry4Aa, Cry4Ba, Cry10Aa, Cry11Aa, Cyt1Aa, and Cyt2Ba toxins [12].

All four types of proteins have been proposed for use as crop protection agents, although Cyt toxins have not as yet been used in commercial insect-resistant transgenic plants, and three-domain Cry toxins are by far the most commonly used type. Cry and Cyt toxins belong to the class of proteins referred to as bacterial

pore-forming toxins, and show structural similarity to the α-helical and β-barrel groups of toxins, respectively (where α-helical and β-barrel refer to the structures of the membrane-spanning parts of the toxin; reviewed by Parker and Feil [13]).These pore-forming toxins show common features of activity; they are produced as water-soluble proteins, and interact with specific receptors on cell surfaces, often after proteolytic activation by host proteinases. Binding to cell surfaces triggers a conformational change leading to oligomerization, which allows insertion into the cell membrane through promotion of a fluid, partially denatured structure. Insertion of the toxin into the membrane can either cause cell death directly, or result in effects on intracellular metabolism which lead to cell death.

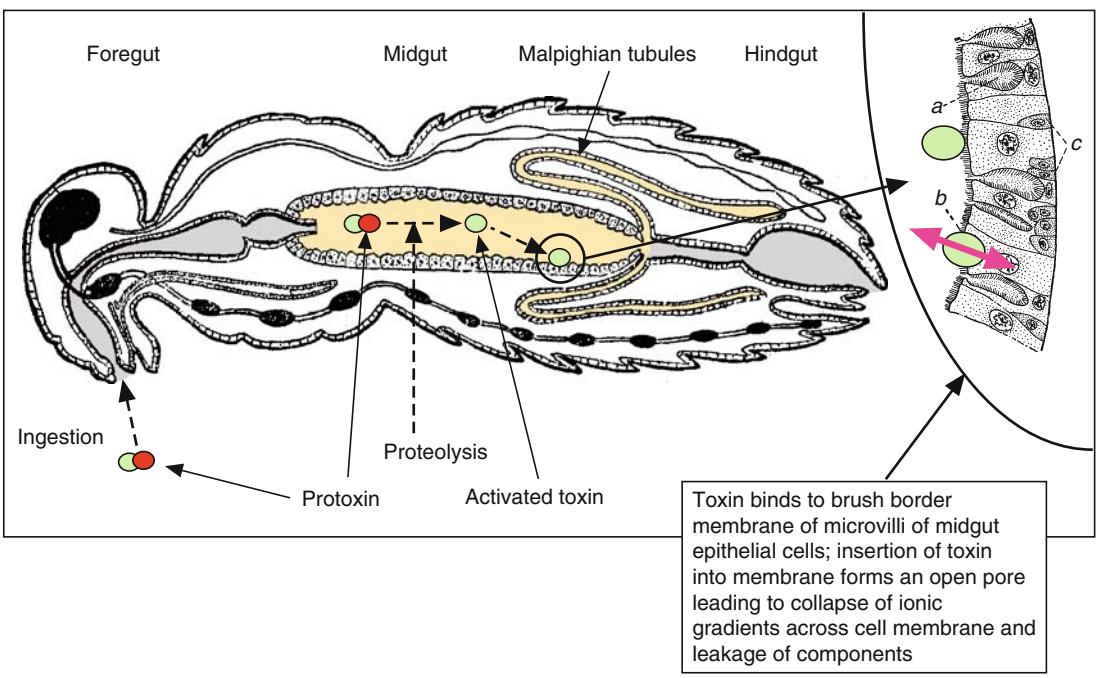
How Do *Bt* Toxins Work?

Three-Domain Cry Toxins

The mechanism of action of the "conventional" three-domain Cry toxins is now well understood, and can be divided into four stages:

1. Solubilization of the protoxin, and proteolytic activation by proteinases in the insect gut to produce active toxin
2. Interaction of the toxin with one or more receptors on cell surfaces in the insect gut epithelium
3. Oligomerization of the toxin
4. Insertion of the oligomerized toxin into cell membranes, leading to the formation of open pores, and cell death (see Fig. 1)

Following the pioneering work of Ellar's group [14] tertiary structures of six different three-domain Cry toxins are known – Cry1Aa [15], Cry2Aa [16], Cry3Aa [14], Cry3Bb [17], Cry4Aa [18], and Cry4Ba [19]; whereas most structures are for the active form, the structure of Cry2Aa includes the N-terminal pro-region. These toxins all show a high degree of structural similarity, and thus the formulation of a general model for their mode of action is justified. The three domains present in the active forms of these proteins are designated I, II, and III, and are normally contained in a single polypeptide of approximately 600 amino acid residues (in some cases proteolytic cleavages are



Genetic Engineering of Crops for Insect Resistance. Figure 1
Action of *Bt* toxins on the insect gut epithelium. Death of insect results from disintegration of gut epithelium (due to cell death) and proliferation of gut microflora

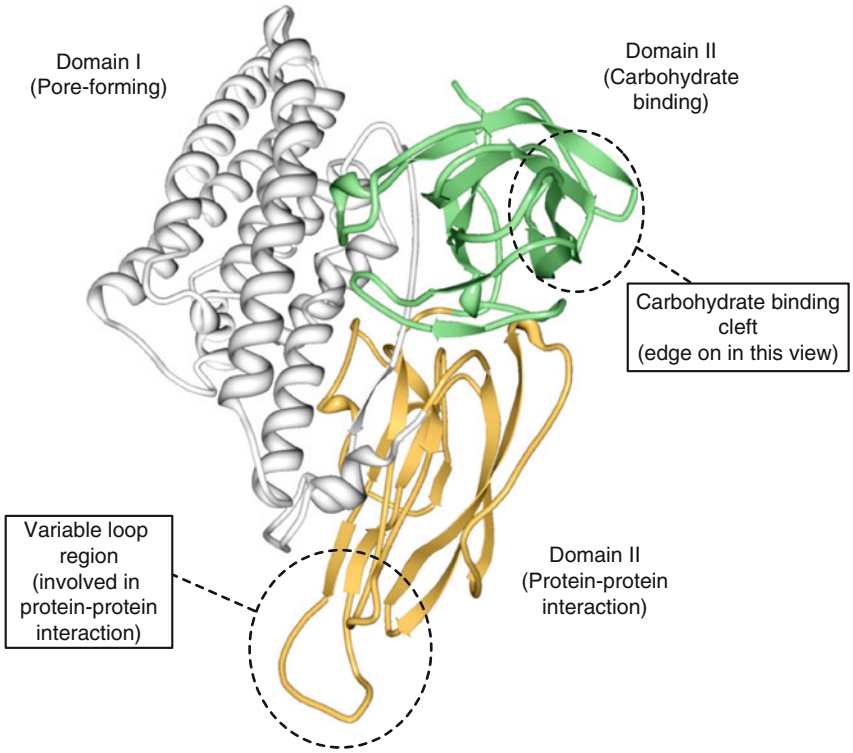
present within the active three-domain structure as a result of protoxin activation, resulting in multiple polypeptides making up the toxin, but the overall three-domain structure is conserved.). While conservation of structure and sequence is observed in the active forms of three-domain toxins, many toxins are synthesized with C-terminal extensions, which are variable in sequence between *Bt* strains, and in length between Cry families. The presence of C-terminal extensions leads to a large degree of heterogeneity in the size of the protoxins present in bacterial spores, with sizes ranging from approximately 600 amino acids (similar to the active toxin) to approximately 1,200 amino acids. These C-terminal extensions are not required for toxin function, and are removed during toxin activation, although their removal is not sufficient for toxicity to be shown. They are thought to play a role in the formation of crystalline inclusions in the bacterium during the spore-forming process.

The three domains of the active toxin are clearly distinguished in their structures.

1. Domain I, approx. 260 aa, contains seven α-helices, of which six are amphipathic and one hydrophobic. This structure is typical of pore-forming toxins, with the hydrophobic and amphipathic helices being responsible for membrane insertion and pore formation. The hydrophilic sides of the amphipathic helices form the surface lining the pore, so that polar species such as ions are able to cross the membrane.
2. Domain II, approx. 170 aa, forms a "β-prism" structure, with three β-sheets, and exposed loops on its surface.
3. Domain III, approx. 160 aa, has a compact structure with two anti-parallel β-sheets in a "jellyroll" formation, and is structurally similar to carbohydrate-binding domains such as the cellulose-binding domain in cellulases [20]. A general model for three-domain toxins is shown in Fig. 2.

The Proteolytic Activation Process Ingestion of the Cry protoxins by the insect leads to solubilization of the proteins, and exposure to digestive proteinases in the insect gut. Although removal of the C-terminal protoxin region occurs at this stage, the essential step in protoxin activation is the proteolytic cleavage and removal of an N-terminal peptide, which varies from approx. 25–60 amino acids in different Cry proteins. A non-activatable Cry1Ac mutant toxin could not form pores in insect membrane vesicles derived from gut epithelial cells [21], and it is thought that the N-terminal peptide "masks" a region of the toxin involved with interaction with receptors [16]. The activated toxin is fairly resistant to further proteolytic cleavage, which enables it to survive long enough in the gut to reach its site of action, the gut epithelial surface (Fig. 1).

This summary overlooks a number of factors which contribute to toxicity. First, the location of the proteolysis may be important, since many insects, such as diptera (flies), carry out digestion in the foregut, which is chitin-lined and does not contain epithelial surfaces, or even outside the insect altogether, by secreted saliva or regurgitated gut contents. Under these circumstances, the toxin will need to be more resistant to proteolysis, or more effective, since the time between activation and reaching the site of action will be longer. Secondly, gut conditions vary significantly between insects from different orders, or even within orders; in general, larvae of lepidoptera (moths and butterflies) have a highly alkaline midgut environment (pH 10–11 in many major crop pests), whereas larvae of coleoptera (beetles) have an acidic gut environment (pH approx. 5 for many species). These differences in conditions will affect both the activation and survival of the protein, although they may be less relevant to steps taking place at the gut surface, where there is a separation from the gut lumen by the peritrophic membrane (a macroscopic porous chitin-based structure) and by lipids sloughed off from the gut surface. Finally, the nature of the digestive enzymes present in the insect gut differs considerably between different orders; whereas most insects use serine proteinases with an alkaline pH optimum as their major endoproteinases, many coleopteran larvae use cathepsin-type cysteine proteinases with an acidic pH optimum (similar to lysosomal proteinases). On the other hand, protoxin activation does not appear to be very sequence specific. Many lepidopteran-specific Cry proteins can be activated in vitro by mild treatment of the protoxin with bovine trypsin, yielding products that appear to be similar to those formed in vivo. This suggests that it is the



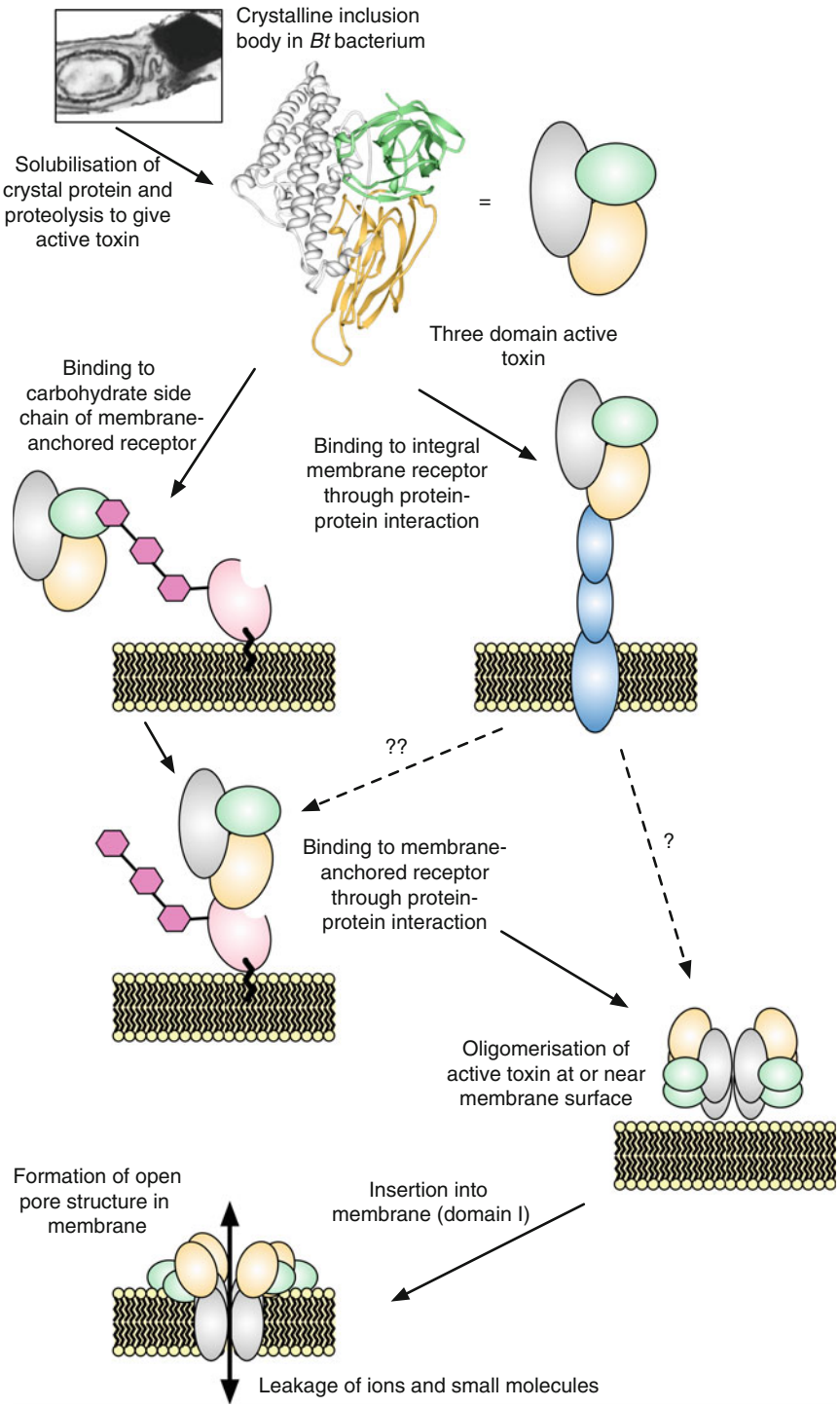
Genetic Engineering of Crops for Insect Resistance. Figure 2
Model structure for three-domain *Bt* toxins. Ribbon diagram showing backbone structure of *Bt* toxin Cry1Aa (PDB 1ciy; [15]); structure of active toxin shown. The three domains are color coded: domain I, silver; domain II, orange, domain III, green. Features as shown on diagram

three-dimensional structure of the protoxin that determines where proteolysis takes place, unless forcing conditions are used.

Interactions with Receptors Proteins to which Cry proteins bind in the insect gut are termed "receptors," although the specificity of interaction is determined by the Cry protein itself, and the ligands to which it binds do not show the properties of receptors as normally understood. Binding takes place on the microvillar membranes of the cells forming the midgut epithelium, and involves interactions with relatively abundant proteins, either attached to the cell membrane by glycosylphosphatidyl-inositol (GPI)-anchors, or integral to the membrane with large extracellular domains. The overall process is summarized in Fig. 3.

Methods for identifying receptors to which Cry proteins bind have largely been based on immunoblotting of proteins prepared from brush border membrane vesicles (BBMV). This method is not a good mimic of conditions in vivo, and may result in interactions with lower affinity, or which are dependent on protein conformations maintained by membranes, not being observed. Nevertheless, the major binding partners for Cry proteins which have been identified show binding when assayed as purified proteins, and as components of BBMVs, with binding constants in the range 1–100 nM.

The initial identification of membrane-anchored aminopeptidase N [23] and an integral membrane cadherin-like protein designated *Bt*-R1 [24] as Cry1A toxin receptors in lepidopteran insects has been supplemented more recently by identification of a 270 kDa glycoprotein [25] and alkaline phosphatase (membrane anchored; [26]) as additional potential receptors. Alkaline phosphatase appears to be the major receptor in mosquitoes [27]. A recent proteomic



Genetic Engineering of Crops for Insect Resistance. Figure 3

Mechanism of action of three-domain *Bt* toxins. The scheme shown is adapted from the "two-receptor" model [22]

analysis has identified further potential receptors, such as V-ATP synthase subunit 1 [28]. However, this analysis also showed binding to actin, which could not be present at the cell surface, showing that results from blotting experiments need to be interpreted critically.

Functional roles as "receptors" for aminopeptidase N and cadherin *Bt*-R1 in Cry protein toxicity are supported by numerous studies. Strains of lepidopteran insects resistant to Cry1 toxins have been identified which show mutations in the gene encoding *Bt*-R1, leading to the production of a truncated cadherin lacking the extracellular domains [29, 30]. The correlation with loss of function of cadherin with loss of susceptibility to Cry toxins suggests that binding to the extracellular domains of cadherin is a necessary step for toxicity. Binding of Cry1A toxin to the cadherin extracellular domains has been demonstrated in vitro, and the binding regions have been identified in some detail [31]. Both gain-of-function and loss-of-function assays have been used to provide further evidence for involvement of cadherin in toxicity; when transiently expressed in mammalian cells that were not normally susceptible to Cry toxin, Bt-R1 genes from silkworm conferred sensitivity to Cry1A toxins [32]; whereas suppression of cadherin expression by RNA interference in tobacco hornworm (*Manduca sexta*) decreased sensitivity to Cry1Ab toxin [33]. In the case of aminopeptidase N, similar correlations between resistance to *Bt* toxin and lack of expression of specific isoforms of the protein have been observed [34], but more direct evidence has come from downregulation of aminopeptidase by RNA interference using double-stranded RNA. This technique has been carried out in lepidopteran larvae, giving decreased sensitivity to Cry1C toxin [35], and in lepidopteran cell cultures, giving decreased sensitivity to Cry1Ac [36]. A gain of function experiment in which transgenic fruit flies (*Drosophila*) expressing lepidopteran aminopeptidase N became sensitive to the lepidopteran-specific toxin Cry1Ac [37] showed elegantly and convincingly that this receptor plays a key role in toxicity. Binding to aminopeptidase N involves interaction of Cry toxins with the carbohydrate side-chains of the protein [38, 39], with specificity toward GalNAc residues being shown (this sugar can inhibit binding; [40]). Binding to carbohydrate facilitates subsequent protein–protein interactions, which are thought to be necessary for toxicity [41]. Functional evidence for alkaline phosphatase acting as a Cry toxin receptor has again been provided by correlative observations, in that insect lines resistant to Cry1Ac toxins have lower alkaline phosphatase levels than susceptible lines [26]. Interactions with protein-bound carbohydrate also seem to be involved in the binding of Cry toxins to alkaline phosphatase.

The roles of the different domains of Cry proteins in the interaction with receptors are clearly distinguished. Despite the presence of the N-terminal propeptide which must be removed for activity, domain I plays little or no role in the interaction with receptors, whereas domain II is responsible for most protein–protein interactions (see Fig. 2), and domain III is responsible for binding to carbohydrates. This division of roles is consistent with the observation that a single toxin can interact with more than one type of "receptor"; for example, Cry1Ac interacts with both Bt-R1 and aminopeptidase N [22]. The protein–protein interactions mediated by domain II have been localized to variable loop regions on the surface of the domain, whereas the carbohydrate-binding region of domain III is a typical binding site cleft, which is spatially well-separated from the domain II loops.

Oligomerization Oligomerization is a common feature in bacterial pore-forming toxins, and Cry proteins appear to conform to the model, with the formation of oligomeric structures (probably tetramers) observed for toxins from the Cry1 and Cry3 families. Mutants of the Cry1Ab protein that have impaired oligomerization ability, but bind to the receptor, show much reduced toxicity or no toxicity toward lepidopteran larvae [42]. Similarly, monomeric Cry proteins have much lower intrinsic pore-forming abilities on synthetic membranes than oligomerized preparations [43]. Oligomerization is promoted by binding to a receptor; in the case of Cry1Ab protein binding to the cadherin Bt-R1 receptor, this process involves an additional proteolytic cleavage at the N-terminal end of the protein, in domain I [44]. The proteolytic cleavage, carried out by host enzymes, may aid the oligomerization process. The importance of oligomerization in promoting toxicity has been shown by two complementary studies. First, a peptide corresponding to the region of cadherin to which Cry1A binds has been shown to act as a synergist, increasing the toxicity of

Cry1A toward lepidopteran larvae [45], presumably as a result of the binding between the peptide and Cry1A promoting oligomerization of the toxin prior to interaction with the gut epithelium. Secondly, mutants of Cry1Ab toxin have been produced which contain deletions corresponding to the proteolysis in helix 1 of domain I which occurs on binding to cadherin. These mutated toxins form oligomers in the absence of cadherin binding, and are effective against insects that have cadherin expression suppressed, or which have a cadherin mutation which leads to resistance to unmodified toxin [33]. These results have led to a current view that cadherin is the primary receptor for Cry toxins, since it is necessary to promote oligomerization, with other molecules taking the role of "secondary receptors" [33].

Insertion into the Cell Membrane The oligomeric Cry protein must partially unfold in order for the pore-forming domains (domain I) to insert into the membrane. In the case of bacterial pore-forming toxins active against mammalian cells, this partial denaturation process is stimulated by acidic pH at the cell surface [13]. A similar mechanism could occur with Cry proteins active against lepidopteran insects, although the gut pH is very alkaline; the partial denaturation could still be triggered by a decrease in pH at the cell surface. The pH optimum for aminopeptidase N in lepidopteran larvae (8.0; [46]) is at least 2 pH units less than bulk gut content pH (>10), suggesting that a decrease in pH occurs near the cell surface. The involvement of lipid rafts, microdomains which are less fluid than the membrane as a whole, in pore formation has been suggested [47]. However, membrane-anchored proteins are selectively associated with these lipid rafts, and it is not clear whether lipid rafts are necessary for pore formation, or whether their involvement is a result of the presence of receptors. The trans-membrane cadherin-like *Bt*-R1receptor is not associated with lipid rafts.

A current model for pore formation by Cry1A toxins suggests that interaction with two receptors is necessary; an initial binding step with the cadherin-like *Bt*-R1 receptor leads to toxin oligomerization, followed by interaction of the oligomer with the aminopeptidase N receptor and insertion into the membrane [22, 48]. While this model is plausible, the details of the mechanism of toxicity must differ for different toxins, and a "two-receptor" model should not be assumed to be generally applicable. The gain of function experiments described above show that only one receptor is necessary for toxicity to be shown, and only a few lepidopteran-specific Cry toxins have been shown to interact with cadherin-like proteins [49]. If the major determinant of Cry protein toxicity is the assembly of oligomeric complexes at the surface of cells in the gut epithelium, then this requirement can be met in diverse ways, involving different "receptor" proteins to localize the toxin and promote oligomerization (although the interaction is always likely to involve the most abundant proteins at the cell surface). A "global" diversity of interactions is not inconsistent with specificity when interactions between specific toxins and hosts are considered.

Once the insertion of Cry toxin into the cell membrane leads to pore formation, the gut epithelial cell is unable to maintain its internal solute balance, as the open pore allows free exchange of ions and other small molecules between the gut lumen and the cytoplasm. The cytoplasm of gut cells has markedly different concentrations of ions (including H^+) than the gut lumen; this difference in concentrations is used to drive active transport processes, such as amino acid transport [50]. Free movement of ions thus causes massive disruption to cell physiology, leading to death. The leakage of cell contents also causes proliferation of gut microflora, so that dying insects show massive bacterial infection of collapsing gut tissue. Cry proteins may also produce toxic effects through interference with signaling pathways. Binding of Cry1Ab to the transmembrane *Bt*-R1 receptor has been shown to activate a G-protein-mediated intracellular signaling pathway, resulting in the formation of cAMP by adenylyl cyclase, and activation of protein kinase A [51]. This process led to cytological changes typical of *Bt* toxin activity.

Binary Cry and Vip Toxins

The binary Cry toxins are exemplified by toxins active against corn rootworm [52]. These toxins are only active as a combination of two proteins, designated as families Cry34 (14 kDa protein) and Cry35 (44 kDa protein). The two proteins are the product of a single

operon in the commonly used *Bt* strains. The binary toxin acts on the insect gut epithelium, and leads to swelling and vesicle production from epithelial cells, resulting in the disappearance of microvilli, and extensive disruption of the epithelium. However, it is not clear whether these symptoms are solely a result of open pore formation, or whether other modes of toxicity, such as ADP-ribosylation (see below) are occurring. No structural information on these proteins is available at present. There is evidence that the 44 kDa toxin protein Cry35 is evolutionarily related to an insecticidal toxin from *Bacillus sphaericus* [53]. The *B. sphaericus* toxins have received some attention due to their toxicity toward mosquitoes and other dipteran insects. They also bind to membrane-anchored receptors (α-glucosidase, in the case of the mosquito *Culex pipiens* [54]) and cause disruption of the gut epithelium [55]. However, their detailed mechanism of action is not known. Like Cry34/35, the *B. sphaericus* proteins are binary toxins, although in this case one component does show limited activity in the absence of the other. The designation of the corn rootworm binary *Bt* toxin by the symbol Cry obscures the fact that these toxins have little in common with the three-domain toxins, besides being found in crystalline deposits in *Bt*, and being insecticidal as a result of acting on the insect gut epithelium.

The *Bt* insecticidal Vip1/2 proteins (active against corn rootworm) are also binary toxins with similarity to the *B. sphaericus* toxins [56]. The mechanism of action of Vip1/2 toxins involves ADP-ribosylation by the active component, which disrupts actin polymerization in cellular microfilaments, similar to other bacterial ADP-ribosylating toxins such as botulinum toxin [57]. The inhibition of actin polymerization leads to massive disruption of cellular functions. The Vip1Ac binding component of the binary toxin interacts with membranes to form oligomeric channels, allowing the active component to gain access to the cell cytoplasm [58].

A further class of Vip proteins, Vip3, (active against lepidoptera) has been identified; these protein are single chain toxins which lyse insect gut cells by pore formation in membranes, and have no sequence similarity to Vip1/2 [59, 60]. Vip3 binds to brush border membrane vesicles prepared from target insect gut epithelial cells, but does not bind to the same receptors as Cry1 and Cry2 proteins [61]. Binding to 80 and 100 kDa membrane proteins is observed in ligand binding experiments [62], but these receptors have not been characterized. These proteins are promising candidates for further development; chimeric toxins containing regions from different Vip3 toxins have been produced and show extended ranges of toxicity toward lepidopteran pests [63].

Cyt Toxins

The cytolytic Cyt toxins, also found in crystalline inclusions in some *Bt* strains, are single polypeptides, of approx. 250 amino acids; the N-terminal region contains α-helices which wrap around a C-terminal β-sheet core in the three-dimensional structure [64]. Pore formation results from insertion of the β-sheet region into membranes [65]. Unlike the three-domain Cry toxins, this membrane insertion is not receptor-mediated [66]; the Cyt toxins insert directly into membranes, and are thus cytolytic to a wide range of cells. Like the three-domain Cry toxins, Cyt toxins are synthesized as inactive protoxins which are activated by proteolysis. Activation involves removal of propeptides from both the N- and C-termini of the protoxin; in the case of Cyt2Aa, 32 aa are removed from the N-terminus and 15 aa from the C-terminus to generate active toxin [67]. This process does not require specific proteinases.

The combination of Cry and Cyt toxins found in crystalline inclusions in some *Bt* strains, specifically in the strains of *Bt* subsp. *israelensis* active against mosquito larvae, is highly effective as a toxin due to synergistic interactions between its components. Not only are the three domain Cry protein components in these crystals more effective toxins in the presence of Cyt proteins, but the Cyt proteins also prevent resistance to Cry proteins from developing when insects are exposed to purified protein preparations under laboratory conditions [68]. This synergistic effect could result from the two types of toxin producing complementary disruption of the insect gut epithelial cell membranes, but evidence has been presented that Cry and Cyt toxins can interact directly. Specifically, Cry11Aa and Cyt1Aa bind strongly to each other, both in solution and in a membrane-bound state, and binding of Cry11Aa to mosquito gut epithelial cell

membranes was enhanced by pretreating the membranes with Cyt1Aa [69]. The interaction with Cyt1Aa takes place through the loop region in Cry11Aa involved in protein–protein interactions with its "normal" receptor (membrane GPI-anchored alkaline phosphatase). Insertion of Cyt1Aa into gut cell membranes, which is not dependent on receptor mediation, thus generates additional "receptors" for Cry11Aa, increasing its toxicity, and preventing resistance developing by mutation of the insect-encoded "receptor."

Expression of Genes Encoding *Bt* Insecticidal Proteins in Transgenic Plants

Expression of Three-Domain Cry Toxins from Transgenes in the Nuclear Genome

Almost all the insect-resistant transgenic crops currently in use express three-domain Cry proteins from *Bt* as their protective agent. The initial laboratory-based experiments expressed Cry1 toxins in plants to give protection against lepidopteran larvae, and this has remained the main focus of *Bt* gene utilization up to the present day. However, the three-domain Cry proteins pose a number of problems in terms of expression in plants. The technology involved in achieving sufficient levels of accumulation of these proteins to give adequate levels of protection was initially challenging, but developed rapidly, so that within 5 years of the initial reports of engineered resistance, the methodology for gene manipulation was essentially complete. The slower pace of transfer of this technology into major crop species observed subsequently has had much more to do with technical difficulties in plant transformation (particularly regenerating viable plants), than with any problems at the level of gene constructs. The minimum level of Cry protein expression in leaf tissue to give high levels of mortality of sensitive lepidopteran larvae under laboratory conditions is approximately 0.05% of total protein, but to give effective field protection against species which are less sensitive to *Bt* toxins, and to manage resistance to the toxin in pests (see later), levels of expression an order of magnitude higher (i.e., 0.5% of total protein) are desirable.

Engineering genes encoding three-domain Cry proteins for expression in transgenic plants has been extensively described (the review by Mazier et al. [70], gives a particularly comprehensive survey), but a short summary of the main considerations which had to be taken into account is relevant here. These were:

1. How much of the protein coding sequence should be expressed in plants?
2. Which promoters should be used to drive expression of the Cry protein coding sequence in plants?
3. How should the coding sequence be altered to avoid poor expression?

Protein Coding Sequence The C-terminal part of protoxins for three-domain Cry proteins is variable, and absent in some toxins. Its role in directing the formation of crystalline inclusions in *Bt* sporulation is not required when the proteins are expressed in plants (and might result in disruption of cells unless the protoxin was exported into intracellular spaces). All constructs which result in insecticidal activity have omitted this part of the molecule from the coding sequence expressed in plants. The initial research suggests that a complete protoxin accumulates in plant tissue at levels 10–50-fold less than a protoxin truncated so the C-terminal region is absent [3]. Since removal of the C-terminal region of the protoxin does not result in active toxin being produced, retention of the N-terminal activation peptide ensures that the initial protein product in transgenic plant tissue is not active, and proteolytic activation takes place as normal within the gut of insect herbivores. The coding sequence utilized thus corresponds to the three-domain structure shown in Fig. 2, plus the additional N-terminal propeptide.

Promoters The problems experienced in achieving levels of expression of Cry proteins high enough to confer effective protection meant that the initial use of promoter sequences which only gave low levels of expression, such as those from *Agrobacterium tumefaciens* Ti plasmids, was rapidly superseded by strongly expressed promoters, most of which were based on the Cauliflower Mosaic Virus 35S RNA promoter (CaMV 35S). Constitutive expression of the Cry protein in all plant tissues does not appear to cause significant problems either in a yield penalty, or

deleterious effects due to the accumulated protein. However, tissue-specific promoters have also been used, such as the ribulose-bisphosphate carboxylase small subunit promoter (e.g., [71]) or the phosphoenolpyruvate carboxylase promoter (e.g., [72]), both of which are specific for green tissue. The CaMV 35S promoter was initially considered to be specific for dicots, but further experience showed that it could also be functional in monocots, and, with suitable modification, could be used to direct Cry protein expression (e.g., [73]). However, many researchers have preferred to use promoters derived from constitutively expressed monocot genes in Cry protein expression constructs for use in cereal transformation (e.g., the maize ubiquitin-1 promoter; [74]). Root-expressed promoters have been used in constructs designed to protect cereals against corn rootworm [75].

Considerable research has also been undertaken on the use of promoters whose expression is only induced under specific conditions. The use of wound-induced promoters to direct Cry protein expression has the apparent advantage that production of Cry proteins in transgenic plants is, for the most part, only induced on attack by insect pests. Any potential deleterious effects on phenotype caused by production of the toxin in transgenic plants would therefore be minimized, and toxin residues in plant tissues would be reduced. A wound-inducible maize proteinase inhibitor gene promoter has been used to direct expression of Cry1B in transgenic rice, and has been shown to give effective protection against insect attack (against striped stem borer; [76]). However, the protection afforded by transgene constructs containing wound-inducible promoters is lower than when constitutive promoters are used, both in the laboratory and in the field [77].

While achievement of expression levels of *Bt* toxins sufficient to confer protection in transgenic plants is now considered routine, considerable technical problems may still need to be overcome when specific crop species are considered (e.g., soybean; [78]). These include the construction of the synthetic coding sequence for the toxin, choice of an appropriate promoter for the expression construct, developing protocols for efficient transformation and regeneration of the plant species, and production of homozygous progeny lines containing the transgene.

Engineering the Coding Sequence to Optimize Expression The initial experiments in which Cry toxins were produced in transgenic plants showed that only low levels of Cry protein were accumulated, generally of the order of 0.01% of total protein, or less. Levels of Cry proteins were at least one order of magnitude lower than when plant proteins were expressed using similar promoters in expression constructs, leading to the deduction that the Cry protein coding sequence contained features which decreased protein production as a result of posttranscriptional events. Cry protein coding sequences are generally A-T rich compared to plants (coding%GC in *Bacillus thuringiensis*, 36%; in *Arabidopsis thaliana*, 45%; in *Oryza sativa*, 55%; Codon Usage Database, http://www.kazusa.or.jp/codon/) and codon usages thus differ significantly. Cry protein genes were reengineered, modifying the nucleotide sequence without altering the encoded amino acid sequence, to change the codon usage to one more appropriate for plants, resulting in either partially or wholly synthetic genes (reviewed by Mazier et al. [70]). Codon optimization for both dicots and monocots has been carried out. Codon-optimized synthetic genes show accumulation levels of Cry proteins of up to 1% of total protein in leaf tissue, which is adequate for complete protection of plants against pest insects [79].

The basis for poor expression of Cry proteins in transgenic plants has received comparatively little attention. Evidence suggests that the major problem is not codon usage, but instability of RNA transcripts [80, 81]. Expression of unmodified Cry protein coding sequences leads to accumulation of short, polyadenylated transcripts resulting from incorrect recognition of polyadenylation addition signal sequences within the protein coding sequence [82]. Specific modification of A-T-rich regions within the coding sequence of Cry1Ac toxin putatively responsible for transcription termination and polyadenylation (both AATAAA signal addition sequences and ATTTA upstream motifs) has been shown to lead to increased protein expression in transgenic tobacco [83]. Changing codon usage to increase GC content has eliminated these A-T-rich regions in synthetic Cry protein genes, which therefore can produce high levels of stable mRNA.

Expression of Three-Domain Cry Toxins from Transgenes in the Chloroplast Genome

The bacterial origin of the chloroplast is reflected in differences in both the genome composition and organization, and the biochemistry of transcription and translation within the organelle, compared to the nuclear genome and transcription and translation in the nucleus and cytoplasm. The bacterial origin of the genes encoding Cry proteins suggests that expression in the plastid, from transgene constructs introduced into the plastid genome, might result in high levels of protein production. This prediction was confirmed in 1995 with a report showing that incorporation of a construct containing a complete coding sequence for the Cry1Ac protoxin protein and the plastid rRNA operon promoter into the genome of tobacco chloroplasts led to accumulation of Cry1Ac protoxin (approx. 130 kDa – i.e., with the C-terminal crystal-forming region intact) in tobacco leaves to levels of 3–5% of total protein [84]. The high level of Cry protein accumulation meant that transformed plants were effectively protected against attack by several major lepidopteran pests, even beet armyworm (*Spodoptera exigua*), a species relatively insensitive to *Bt* toxins.

Despite this highly promising initial report, expression of Cry proteins via plastid transformation has not been widely adopted, and is not used in the current commercial crops. Reasons for this are difficult to pinpoint; there are significant technical problems in achieving stable transformation of plastids, since all of the copies of the plastid genome in the cell (up to 10,000) must be transformed [85], and plastid transformation has been problematic in species other than tobacco [86]. Nevertheless, methods exist to overcome these problems [87]. Cry1, Cry2, and Cry9 proteins have been expressed in plastids of tobacco [88–91], and Cry1Ab has been expressed in soybean plastids [92], all giving high levels of protection against lepidopteran pests to the resulting plants. Overexpression of the Cry2Aa2 operon is particularly effective in giving broad-spectrum protection against a range of pests.

Commercial introduction of transgenic insect-resistant crops based on plastid transformation is almost certainly feasible, but may as yet be restricted by economic considerations, or concern over long-term stability of the transgene phenotype. The maternal inheritance of plastid-encoded characteristics shown by most plants, which means that pollen cannot disperse the transgene to non-transgenic plant stocks, is a further advantage to the method, which could be used to overcome objections to coexistence of transgenic and "organic" agricultural practices by environmental pressure groups.

Expression of Other Genes Encoding Insecticidal *Bt* Toxins

Gene constructs for expression of other *Bt* toxins follow the same principles as those outlined above for three-domain Cry toxins. For example, corn expressing the binary Cry34/35 toxin (for protection against corn rootworm) was transformed with a construct containing a constitutive promoter (maize ubiquitin-1) and synthetic coding sequences for the 44 and 14 kDa polypeptides [52], giving expression levels of up to 0.9% and 0.2% respectively of total soluble proteins in plant tissues. Details of the constructs used for expressing these, and other *Bt* toxins, are apparently not reported in the scientific literature.

Taking Transgenic Plants Expressing *Bt* Toxins into the Field

Dealing with Pest Resistance to *Bt* Toxins

The development of successful strategies for commercial deployment of "first generation" insect-resistant crops expressing a single three-domain Cry toxin has focused on a single major potential problem, the development of resistance to the insecticidal compound by the targeted pest species. Development of resistance to exogenously applied chemical pesticides has occurred in over 500 insect species [93], and field resistance to *Bt* sprays has been observed in the lepidopteran pest diamondback moth (*Plutella xylostella*). Resistance to *Bt* toxins can be produced in the laboratory within a small number of generations of many pests, showing that resistance alleles are present in pest populations at a nonnegligible level, although resistance to high doses of specific toxins is only shown in individuals homozygous for the resistance allele. This topic has been ably

reviewed in the context of the commercialization of *Bt* crops by [94]. The most common mechanism of resistance to Cry toxins in insects is mutation in a toxin receptor, leading to a failure to bind sufficient levels of toxin for lethal effects to be shown; however, the involvement of more than one "receptor" in current models for three-domain Cry toxin mechanisms of toxicity (see above) implies that multiple genetic loci for resistance in the pest are possible. Other mechanisms, such as altered proteolysis of toxins, have been proposed to account for the resistance to multiple toxins which can be produced in the laboratory.

The practical solution to prevent the development of resistance in pest populations, the "high-dose/refuge" strategy, has been extensively reviewed elsewhere [94]. In its simplest form, this strategy couples transgenic plants that are expressing sufficient levels of a specific toxin to kill all pest insects which are homozygous negative, or heterozygous, for a resistance allele, with a reservoir of untransformed plants which maintain a population of pests which have a normal frequency of resistance alleles. It assumes that the frequency of occurrence of resistance alleles is low ($<10^{-3}$). Surviving pests on the transgenic plants will be almost all homozygous positives for the resistance alleles, but will be few in number due to the low frequency of occurrence of these alleles. The nontransformed plants will produce a large number of pest insects, most of which are homozygous negative for resistance alleles. Provided that transgenic and untransformed plants are not spatially separated, mating between resistant insects selected on transgenic plants will be a rare event, and most progeny will be homozygous negative or heterozygous for resistance alleles, and thus susceptible to the insecticidal activity of the transgenic plants. In this way, both the pest population is suppressed, and any increase in the frequency of resistance alleles in the population is minimized by the continuous "diluting out" effect.

This approach has been almost wholly successful in controlling pest resistance to *Bt* toxins in agricultural use of transgenic crops over 10 years. That it has been so successful may be a result of factors other than those originally considered, since the assumption that *Bt* toxin resistance alleles occur at a very low frequency in natural populations has been called into question. Although some insect populations show resistance allele frequencies in the 10^{-3} to 10^{-2} range (e.g., tobacco budworm, *Heliothis virescens* in USA; [95]; *Sesamia nonagrioides* in Spain and Greece; [96]), estimates for pink bollworm (*Pectinophora gossypiella*) in Arizona, USA in 1997 were as high as 0.16 [97]. No evidence for selection for resistance was observed, since the frequency of resistance alleles did not increase over a 3-year monitoring period in which transgenic cotton expressing *Bt* toxins was extensively employed. A subsequent follow-up study [98] confirmed that frequencies of resistance alleles in this insect had not increased over an 8-year monitoring period, with values generally $<10^{-2}$, despite almost continuous exposure to Cry1Ac via transgenic cotton. The possibility that resistance alleles in the insect carry a significant fitness penalty is one additional factor that could account for these observations.

The success of the refuge strategy is dependent on farmers sacrificing part of their crop (untransformed plants) to maintain a pest population. This has been successfully enforced in the industrialized agriculture of developed countries, but may be more difficult to ensure when insect-resistant transgenic crops become available to rural farmers. Although greater agricultural diversity may play the same role as the refuge strategy in maintaining a pest population and decreasing selection pressure, emergence of resistance in pests to *Bt* crops has been delayed, not eliminated, and further strategies to manage it will be necessary.

Pests That Are Not Susceptible to *Bt* Toxins

As described above, most of the *Bt* toxins that have been investigated, and introduced into transgenic crops, are active against lepidopteran or coleopteran insect pests. This is partly a result of the practical requirements of agriculture, since these orders include most of the major pests. However, there are significant insect herbivores which remain outside the range of activity of *Bt* toxins that have been expressed in transgenic plants.

Dipteran pests, such as fruit flies and root flies, are serious pests in many crops, and *Bt* toxins active against diptera have been thoroughly investigated. A major problem with introducing protection against these pests into plants is that *Bt* strains active against dipteran insects usually contain a mixture of toxins, often

including both Cry and Cyt proteins (see above). These toxins act synergistically, and individual components are only of low toxicity. Introduction of genes encoding the mixture of toxins found in a typical dipteran-active *Bt* strain into a transgenic plant has yet to be attempted, although it is not beyond the capacity of existing technology.

The major order of insect herbivores outside the range of *Bt* toxins is Hemiptera, which includes aphids, plant- and leafhoppers, whitefly, and other sap-suckers which feed directly on the contents of phloem and/or xylem vessels, predominantly sucrose and free amino acids. These insects are important pests and virus vectors. No *Bt* toxins with activity against them have been found. The reason for this is not clear; receptors similar to those in other insect orders are present in these insects [99], but generally they contain very low levels of digestive proteolytic activity, as a result of ingesting nitrogen in the form of amino acids rather than protein. This lack of digestive proteolytic activity may interfere with activation of *Bt* toxins, and prevent enough activated toxin to have effects on the insect being present in the gut.

Why Haven't Plants Evolved Their Own *Bt*?

Despite the problems encountered in managing resistance of pests to *Bt* toxins, transgenic plants expressing these insecticidal proteins have proved their value in the field. However, the necessity for resistance management suggests that this solution to defense of plants against insect herbivores may not be viable on an evolutionary timescale. Endogenous expression of *Bt* toxins is not a "natural" method of defense against herbivores, since plants do not produce similar insecticidal proteins themselves. This failure on the part of plants to exploit a viable strategy for protection seems puzzling, and the obvious explanation, that plants lack the capacity to produce *Bt* toxin-like proteins, is not correct. Since introduction of suitably modified *Bt* genes gives adequate levels of protein expression for protection, there is no reason why plants could not have evolved a similar capacity. As discussed in the following section, plants have evolved a diverse array of defensive mechanisms, but make little use of proteins which are highly toxic to insect herbivores. Possibly, this is due to the relative ease with which insects can develop resistance to protein toxins which exert a very strong selection pressure on the population; although alternative hypotheses, such as the balance between investing plant resources into defense versus growth not favoring this strategy, or practical difficulties for a sessile organism in delivering toxins, should also be considered. Unfortunately, the experiments which would enable this issue to be investigated, namely, an evaluation of the "fitness" of *Bt*-expressing plants in a natural ecosystem in competition with varieties relying on endogenous defenses, and the persistence of *Bt* genes in a natural population, are unlikely to be carried out in the near future, due to obvious regulatory issues.

Whatever the reason for plants "in the wild" not using defensive proteins similar to *Bt* toxins, there is no reason to suppose that transgenic plants with engineered insect resistance will not continue to be useful in the artificial growing conditions of agriculture. Manipulation of crop plants by conventional breeding has successfully introduced characteristics such as large seed size, which were not present, and would not be viable, in their wild progenitors. Characteristics introduced into cultivated plants by plant genetic engineering do result from a process that is fundamentally different from selection, but both conventional breeding and genetic engineering are aiming for the same end results, agriculturally desirable phenotypes. Their products should be evaluated by similar criteria.

Developments to "First-Generation" Crops Expressing *Bt* Toxins

Plants Expressing Multiple Toxins ("Pyramiding")

The specificity of a single Cry toxin toward specific target pests can be a problem in the field where a secondary, minor pest species can replace the primary pest and cause serious damage to crops. An obvious method to counter this problem is to add or introduce a second *Bt cry* gene into the crop to extend the range of pests against which protection is afforded. The availability of a wide range of gene constructs encoding Cry toxins has made this a realistic possibility, with crossing singly transformed lines, or repeated transformation, or transformation with a construct containing two genes as alternative methods for introducing the

genes into one line. Monsanto's Bollgard transgenic cotton was improved by introducing a second *Bt* gene as early as 1999. Laboratory trials showed that cotton plants expressing both Cry1Ac and Cry2Ab proteins were more toxic to bollworms (*Helicoverpa zea*) and two species of armyworms (*S. frugiperda* and *S. exigua*) than cotton expressing Cry1Ac alone, even though doses in this trial were sublethal [100]. Subsequent evaluations in greenhouse and field trials [101] confirmed the superior insect resistance of plants expressing both toxins.

A further potential advantage of transgenic plants expressing two Cry proteins with differing specificities, that target different receptors in the insect, is in preventing the appearance of resistance in the pest, since multiple mutations are required to produce the loss of sensitivity to the toxins. This hypothesis was confirmed directly in work reported by [102], in which transgenic broccoli plants expressing either Cry1Ac, or Cry1C, or both proteins were produced. Plants were exposed to a population of diamondback moth (*P. xylostella*) which carried Bt resistance genes at a relatively low frequency in an extended greenhouse experiment, and results showed that selection over 24 generations led to a significant delay in the appearance of resistance in insects exposed to the pyramided two-gene plants. The success of these experiments has led to suggestions that the refuge approach to resistance management may be redundant for crops expressing multiple toxins [103]. However, some care is needed in the selection of genes in relation to potential pests, as resistance to multiple toxins has been observed in several cases. For example, a strain of the lepidopteran cotton pest *H. virescens* which has simultaneous resistance to Cry1Ac and Cry2Aa has been identified, in which the genetic bases of resistance to each toxin are different [104].

Many subsequent programs which have aimed to produce insect-resistant crops expressing *Bt* toxins have adopted the two-gene approach to broaden and improve protection against diverse pests, and to prevent resistance developing in insects (e.g., [105]). Although engineering to produce combinations of different three-domain Cry toxins is the most common approach, other potential resistance genes have been included also, such as those encoding Vip proteins [106], or even proteinase inhibitors (e.g., cowpea trypsin inhibitor; [107]). The "pyramiding" or "stacking" of resistance transgenes has been enthusiastically adopted by commercial organizations, and the recent announcement of a transgenic maize variety containing eight different transgenes by Monsanto and Dow Agrosciences [108] exemplifies this trend. This variety contains insect-resistance genes derived from both companies' research programs, active against corn rootworm and lepidopteran pests (Herculex RW = Cry34Ab1 + Cry35Ab1, Herculex I = Cry1F; YieldGard VT Rootworm/RR2 = modified Cry3Bb1, YieldGard VT PRO = Cry1A.105 + Cry2Ab2), as well as two herbicide tolerance genes (giving resistance against glyphosate and glufosinate-ammonium), and is intended to be a "one-stop" solution to pest and weed problems.

Domain Exchange in Three-Domain Cry Toxins

The separate roles played by the different domains in the process of interaction of three-domain *Bt* toxins with their receptors, and their structural independence, suggested to investigators that hybrid toxins, in which domains from different naturally occurring toxins were grafted together, would be likely to be active, and could show novel specificities in their activity toward insects. This process can be made to occur in vivo in *Bacillus thuringiensis*, using a site-specific recombination vector [109], or can be carried out in vitro using conventional molecular biology techniques, followed by expression in a microbial host. Transfer of domain III between different Cry1 proteins led to identification of this domain as conferring primary specificity to different lepidopteran species, and the generation of hybrids with broader specificity than naturally occurring toxins [110]. Subsequent work generated a Cry1Ab-Cry1C hybrid, which was highly toxic to *S. exigua*, an insect resistant to Cry1A toxins [111], and identified Cry1Ca domain III as sufficient to confer toxicity toward *Spodoptera* in a variety of hybrids [112]. In contrast to the results obtained when exchanging domain III, exchange of domain I between different Cry1 toxins did not yield biologically active proteins [113].

A measure of the potential for improvement in "natural" *Bt* toxins is shown by experiments reported by [114], in which a hybrid Cry protein, constructed by fusing domains I and III from Cry1Ba with domain II

of Cry1Ia, was expressed in transgenic potato. Plants expressing the hybrid toxin at levels up to 0.3% of total soluble protein were produced, and not only showed resistance to the lepidopteran pest potato tuber moth (*Phthorimaea operculella*), but also had a high level of resistance to Colorado potato beetle. The "parental" Cry proteins have high toxicity towards lepidopterans, but only very limited toxicity towards coleopterans such as the potato beetle. The hybrid has effectively created a novel toxicity, which is suggested to be based on interaction with a novel receptor.

Mutagenesis of Three-Domain Cry Toxins

Modification of *Bt* toxins by site-directed mutagenesis to increase toxicity towards target pests has been employed as an alternative to the "domain swap" approach. Most mutagenesis experiments on *Bt* toxins have been carried out to explore structure-function relationships in these proteins (see above; reviewed by Dean et al. [115]), but the accumulated knowledge of which parts of the protein determine specificity of interactions with receptors in the insect have been exploited to produce variants with increased activity toward target pests.

The key role of domain II in three-domain Cry proteins in mediating interactions with insect receptors was shown by a mutagenesis experiment in which altering amino acid residues in the loop regions in this domain of Cry1Ab increased its toxicity toward larvae of gypsy moth (*Lymantria dispar*) by up to 40-fold, with a corresponding increase in binding affinity to brush border membrane vesicles [116]. These results were based on expression of the recombinant protein in microbial hosts. A similar strategy was used to increase the toxicity of Cry3A protein toward target coleopteran pests [117], and of Cry4Ba toxin [118, 119] and Cry19Aa toxin [120] toward mosquito larvae. The level to which rational design of toxins is possible is shown by the engineering of toxicity toward mosquito larvae into the lepidopteran-specific toxin Cry1Aa [121]. Alternatively, a directed evolution system based on phage display technology for producing toxins with improved binding to a receptor, and thus increased toxicity, has been described [122]. Mutagenesis of domain I has also been attempted, with claims that alteration of alpha helix 7 in Cry1Ac to resemble the corresponding helix in diphtheria toxin led to increased toxicity toward cotton bollworm (*Helicoverpa armigera*) larvae [123].

The impressive achievements of toxin engineering at the level of recombinant proteins, have led to the technology being used for gene constructs designed for expression in transgenic plants, although toxins with unmodified amino acid sequences continue to be widely used (largely as they give adequate protection). One example where toxin engineering has been successfully carried out is the current commercial transgenic corn variety with resistance to corn rootworm, MON863, which expresses a modified version of the *Bt* Cry3Bb1 toxin [75]. Unmodified Cry3Bb1 is active against a number of coleopteran species, including Colorado potato beetle and corn rootworm [124], but toxicity toward western corn rootworm (*Diabrotica virgifera virgifera*) was not sufficient to give adequate protection at levels of expression achievable in corn. Modifications to the amino acid sequence increased the toxicity of the protein toward corn rootworm approximately eightfold. The nature of the modifications has not been described in the scientific literature, and is only available through reference to a series of patents (see [75]).

Fusions

As a logical extension to the transformation of plants with separate gene constructs encoding two Cry proteins, some workers have chosen to produce a single construct containing a single translationally fused coding sequence encoding both proteins. This approach has been successfully demonstrated by producing a Cry1Ab-Cry1B translational fusion protein in transgenic maize [125], although there is no apparent advantage over simpler methods for introducing two genes. The Cry1Ab-Cry1B fusion protein has also been expressed in transgenic rice [126], which was fully resistant to yellow stem borer (*Scirpophaga incertulas*).

A more interesting possibility is the introduction of extra functionality into Cry toxins by addition of sequences from other proteins which could lead to binding interactions with more potential receptors in the insect gut, extending the range of toxicity and hindering development of resistance. In work reported by Mehlo et al. [127], the galactose-binding lectin

domain (B-chain) from the ribosome-inactivating protein ricin was fused C-terminally to domain III of Cry1Ac, producing a Cry1Ac-ricin B-chain fusion protein. The fusion protein thus has the ability to bind to galactose residues in side chains of glycoproteins or glycolipids in the insect gut epithelium, as well as *N*-acetyl galactosamine residues which are bound by domain III. The fusion protein was expressed in transgenic maize and rice plants, and was shown to afford a high level of protection to larvae of stemborers (*Chilo suppressalis*) and leaf armyworm (*Spodoptera littoralis*), whereas plants expressing the unmodified Cry1Ac were susceptible to both insects. The transgenic maize plants were also resistant to a homopteran plant pest, the leafhopper *Cicadulina mbila*, although it is possible that this was an effect of the lectin domain in the fusion (see later section Lectins), since *Bt* toxins are not effective against homopteran insects.

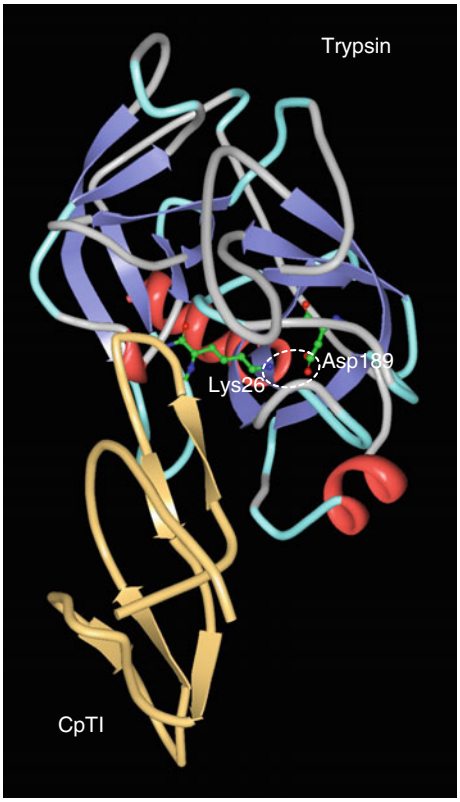
The engineering of extended binding properties into three-domain Cry proteins to increase the range of toxicity toward insect pests is clearly possible, but needs to be approached with some caution. There is a risk that the extended range of activity will include mammalian toxicity, which would negate one of the major advantages of these insecticidal proteins.

Exploitation of Endogenous Plant Defensive Mechanisms Against Insect Herbivores

Plants have a range of endogenous mechanisms to defend themselves against insect herbivores, and use both static defense mechanisms based on the accumulation of pre-synthesized insecticidal compounds, and active defense mechanisms in which gene expression is induced as a result of insect damage (response to wounding, and responses to insect secretions), leading to the synthesis of insecticidal compounds [128]. Conventional breeding has sought to exploit endogenous insecticidal genes within a plant species, but the use of transgenic technology allows defensive compounds and mechanisms to be transferred between species, or allows the control of existing defensive systems to be altered to improve their effectiveness. The molecular biology involved in transfer of genes between plant species is technically straightforward, and does not involve the kind of reengineering necessary to make bacterial genes suitable for use in plants. This approach to increasing insect resistance in transgenic plants has almost as long a history as engineering for *Bt* Cry toxin expression, but to date has not resulted in a commercial product, or widescale adoption in agriculture. Some of the reasons for the lack of practical outcomes for this strategy will be discussed below.

Proteinase Inhibitors

Protein proteinase inhibitors (PIs) are ubiquitous in plant species. They are major components of both "static" and "active" defense in that they are accumulated in specific tissues ("static" defense), and are the major end-product in the induced response to wounding ("active" defense). They are generally small proteins, ranging in size from 4 to 25 kDa, with many different sequence families having been identified. They form tightly bound complexes with their target proteinases, which usually involve a "loop" on the inhibitor fitting into the enzyme active site (Fig. 4), blocking the site, and inactivating the enzyme. The observation that most of these inhibitors were active against digestive serine proteinases from higher animals, and not endogenous plant proteinases (where serine proteinases are comparatively rare, and not involved in protein digestion) suggested that they were defensive compounds, and bioassays in which purified PIs were fed in artificial diet confirmed that an antimetabolic effect was exerted on insect herbivores which relied on protein digestion for nitrogen supply, shown as a slower growth rate, retarded development, and increased mortality (reviewed by Garcia-Olmedo et al. and Ryan [130, 131]). Besides a direct effect on digestion of ingested proteins, PIs cause a loss of nitrogen to the insect by preventing the reabsorption of nitrogen used to produce digestive proteinases, which are normally (self)-degraded in the gut rather than excreted. The role of these proteins in induced defense against insects was shown by blocking the normal wounding response in transgenic tobacco plants by suppression of expression of the prosystemin gene, which produces the peptide hormone systemin, using antisense RNA. The transformed plants were unable to synthesize wound-induced PIs and were significantly more susceptible to herbivory by lepidopteran larvae [132]. The importance of the wounding response to plant defense in natural ecosystems has been



Genetic Engineering of Crops for Insect Resistance. Figure 4

Structure of a complex between a typical plant protein proteinase inhibitor (*PI*) and a target proteinase (from PDB 2g81; [129]). Structure shown in backbone representation is the complex between beta-trypsin (*top*, secondary structure color-coded in red and blue) and a Bowman-Birk PI from cowpea (*Vigna unguiculata*; *bottom*, gold). This inhibitor ("CpTI") has been expressed in transgenic plants to give partial resistance to lepidopteran larvae. The side chains responsible for the specificity-defining ion-pair interaction (dotted ellipse) are shown in ball-and-stick representation; they are Asp189 (S1′) in the substrate binding pocket of the enzyme, and Lys26 (S1) on the active site loop of the inhibitor. Other interactions take place across the contact surface between inhibitor and enzyme to form a tightly bound complex

extensively studied by Baldwin's group (reviewed in [133]); this outstanding body of work has established a synthesis of responses in the plant under attack, responses in neighboring plants, and responses of natural enemies of insect herbivores, with communication via volatile signals produced by the plant under attack.

A seed-expressed Bowman-Birk-type serine proteinase inhibitor from cowpea, which contained two inhibitory sites active against bovine trypsin (CpTI) was the first plant PI to be produced in another species [134], using a gene construct containing a CaMV 35S promoter. The resulting transgenic tobacco plants expressed CpTI at up to 1.0% of total soluble protein, and decreased growth and survival of tobacco budworm (*H. virescens*) by up to 50%, with similar effects on other lepidopteran larvae. Subsequent experiments carried out with wound-induced PIs showed that these also had similar effects when constitutively expressed in transgenic plants; for example, the tomato inhibitor II gene, when expressed in tobacco, was also shown to confer insect resistance [135], as did potato PI-II [136]. Both CpTi and PI-II were subsequently expressed in rice, where partial protection against stem borers was observed [137, 138]. The constitutive expression of foreign PIs could be mimicked in transgenic tomato plants by constitutive expression of the prosystemin gene (see above) leading to constitutive expression of wound-induced tomato PIs [139]. Tobacco plants modified in this manner show partial resistance to insect herbivores similar to that produced by expressing foreign PIs [140].

The problem with this strategy for producing insect-resistant plants soon became obvious; in contrast to the expression of Cry proteins, which, when optimized, routinely gave transgenic plants virtually complete protection against susceptible pests (mortality100%, damage minimal) expression of PIs only produced partial resistance. Investigation of the digestive biochemistry showed that exposure to PIs in the diets of lepidopteran and coleopteran herbivores resulted in the appearance of proteinase activities which were insensitive to the inhibitor(s) present [141, 142], or were able to degrade the ingested PIs [143]. These insects contain large families of genes encoding dietary proteinases, whose expression could be up- or downregulated by dietary inhibitors [144]. In effect, these insect herbivores were preadapted to be partially resistant to dietary PIs, as a result of similar or identical compounds being present routinely in their diet. Although expression of resistance to PIs in herbivorous

insects has a fitness penalty, shown by reduced growth on diets to which inhibitors are added, or on plants which are expressing foreign PIs, or over-expressing endogenous PIs (see above), this is not sufficient to cause mortality at a level which affords more than partial protection. In some cases, low levels of expression of a foreign PI in transgenic plants can actually result in improved insect performance, as when tobacco and *Arabidopsis* plants expressing mustard trypsin inhibitor 2 were exposed to larvae of cotton worm (*S. littoralis*; [145]).

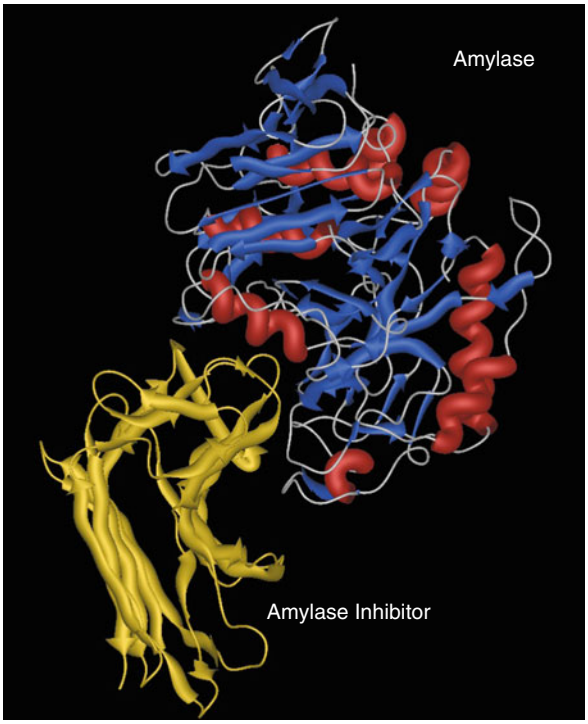
A number of investigators have attempted to select PIs for expression in transgenic plants which are optimally active against the dietary proteinases present in specific insect pests. Attempts to develop inhibitors active against specific lepidopteran digestive serine proteinases induced by dietary PIs have not been successful. On the other hand, not all pest insects rely on serine proteinases for digestion. Many herbivorous coleopteran larvae utilize cysteine proteinases, rather than serine proteinases, as their major digestive endoproteinases, and these proteinases can be inhibited by cystatins, a family of proteins present in all kingdoms of organisms. Enzyme assays in vitro were used to characterize digestive proteinases of a coleopteran pest, *Chrysomela tremulae,* as cysteine proteinases, and to show that a cystatin from rice, oryzacystatin, was an effective inhibitor. Transgenic poplar seedlings expressing oryzacystatin were produced, and leaves from these plants were shown to be toxic to larvae of the pest [146]. This promising result does not seem to have been followed up. Expression of oryzacystatin in transgenic potato only gave partial protection against larvae of Colorado potato beetle [147], suggesting preadaptation in this pest, which is known to employ a diverse range of digestive proteinases. In an attempt to use proteinase inhibitors which insects would not be preadapted to, synthetic multidomain cysteine proteinase inhibitors based on domains found in animal and plant sources (kininogen, stefin, cystatin C, potato cystatin, and equistatin) were assembled and expressed in transgenic potato; the plants were deterrent to thrips, and gave partial resistance in greenhouse trials, but complete protection was not observed [148, 149]. Attempts to express the sea anemone cysteine/aspartic proteinase inhibitor equistatin itself in transgenic potato did not give significant levels of resistance to Colorado potato beetle, due to degradation of the inhibitor in the plant [150]. Multiple proteinase inhibitors (potato PI-II and PCI) active against two families of proteinases, serine proteinases and carboxypeptidases, have been expressed in transgenic tomato plants [151], but still only afforded partial protection against lepidopteran larvae due to adaptive mechanisms present in the insects.

In conclusion, the expression of suitable PIs in transgenic plants can give protection against lepidopteran and coleopteran pests, but has not been able to produce results comparable with those achieved by use of *Bt* toxins.

Amylase Inhibitors

The widespread occurrence of protein inhibitors of mammalian amylases in plants has become accepted as another defensive mechanism against herbivores (reviewed by Franco et al. [152]). Like proteinase inhibitors, these are generally small proteins, resistant to proteolysis, ranging in size from approx. 8–30 kDa. Although they are also active against insect amylases, it is not clear to what extent these proteins contribute to insect resistance in most cases, since the relatively low nitrogen content of plant tissues compared to insects means that most herbivorous insects are nitrogen limited, not carbon limited, and starch digestion is unlikely to be a limiting factor in growth. However, in the case of coleopteran herbivores whose larvae attack seeds specifically, such as seed weevils (bruchids), there is good evidence for α–amylase inhibitors from legume seeds being highly insecticidal [153], and in being causative factors in the resistance of specific varieties of legumes to bruchids [154]. These proteins belong to a different sequence family than the more common types of α–amylase inhibitors found in cereals, and are similar to legume lectins in sequence [155].

Like proteinase inhibitors, amylase inhibitors form tightly bound complexes with their target amylase (Fig. 5), although the same interaction of a loop on the inhibitor with the active site of the enzyme is not possible, since the enzyme substrate is a polysaccharide, not a polypeptide. The mechanism of toxicity clearly involves inhibition of starch digestion, since bruchid larvae exposed to the α–amylase inhibitor from French



Genetic Engineering of Crops for Insect Resistance. Figure 5

Structure of a complex between a plant protein α-amylase inhibitor and an insect amylase enzyme (from PDB 1viw; [156]). Structures shown in backbone representation; α-amylase from larvae of the coleopteran storage pest *Tenebrio molitor* (yellow mealworm beetle) is shown *top right*, in red and blue (secondary structure color coding); the α-amylase inhibitor from *Phaseolus vulgaris* (French bean) is shown in gold *bottom left*. This inhibitor has been expressed in several transgenic legume species to give resistance to coleopteran pests. The inhibitor shows the typical "all β-sheet" structure of the legume lectin family of proteins. Interaction between the binding loop of the protein and the starch-binding site of the enzyme occurs across the contact surface, sterically blocking access by polysaccharides to the active site

bean (*Phaseolus vulgaris*) show induction of amylase enzymes [157], although other mechanisms of toxicity may also be present, since these proteins can cause 100% mortality in susceptible insect species at levels of <1.0% of total protein. Alternatively, these highly specialized herbivores may lack the adaptive mechanisms to plant defensive proteins shown by species that feed on a wide range of plant foodstuffs [128]. High levels of toxicity toward insects have not been observed in general with amylase inhibitors. For example, α–amylase inhibitors are not strongly toxic to lepidopteran larvae, where the alkaline environment of the gut may interfere with the formation of inhibitor-enzyme complexes. The α–amylase inhibitor from French bean is inactivated by high pH.

The isolation of a lectin-like α–amylase inhibitor gene from *P. vulgaris* [155] stimulated research in this area, and in a ground-breaking series of experiments, this gene was assembled into a construct with a strong seed-specific promoter (from the *P. vulgaris* seed lectin gene), and expressed in seeds of transgenic garden pea. The resulting seeds contained up to 3% of the foreign protein, and were highly resistant to larvae of cowpea and Azuki bean weevils [158], which do not normally attack garden peas in the field, but are stored product pests, and to larvae of the pea weevil *Bruchus pisorum* [159], which is a field pest of garden pea. In all cases larval development from eggs laid on seeds was halted at a very early stage, and damage to the crop was minimal. Subsequent experiments showed that transgenic azuki beans could also be protected against bruchid storage pests [160], and that transgenic garden pea was protected against pea weevil under field conditions [161]. The success of this strategy led to hopes that the *Phaseolus* α–amylase inhibitor gene could be incorporated into a range of crops, particularly other grain legumes such as lentils, mungbean, groundnuts, and chickpeas to give protection against a variety of bruchids. Technical problems with transformation of some of these crop species have delayed this goal being achieved, but transgenic chickpeas expressing high levels of the *Phaseolus* α–amylase inhibitor have been successfully produced [162].

Despite the success of this strategy, full agricultural deployment of transgenic crops expressing the *Phaseolus* α–amylase inhibitor gene has not taken place. Commercial reasons have played a major part in preventing widescale adoption, but safety concerns have also arisen. The protein product of the *Phaseolus* α–amylase inhibitor gene expressed in pea shows minor structural differences to the native product (i.e., expressed in *P. vulgaris*) as a result of differences in posttranslational processing (differences in the extent of glycosylation, and in minor components

resulting from proteolysis). Whereas consumption of the native form of the *Phaseolus* α–amylase inhibitor by mice did not result in immunological responses, consumption of transgenic peas expressing this protein led to the presence of circulating antibodies directed against it, and systemic immunological responses including inflammatory responses (i.e., allergic responses) to inhaled or injected protein [163]. In contrast to some earlier work claiming that consumption of transgenic plant material was harmful, this study has been published in a fully peer-reviewed journal and the quality of the research has not been disputed. Further research will be necessary to identify, and remove, the cause of this increased antigenicity. An additional potential drawback was revealed by feeding trials of transgenic peas expressing *Phaseolus* α–amylase inhibitor with pigs and chickens. These trials did not show immunological effects on animal health, but did show that starch utilization by the animals was significantly decreased due to the presence of the inhibitor in the transgenic peas when compared to non-transgenic peas, consistent with the effect of the protein on higher animal amylases [164, 165]. This factor would limit the utility of transgenic peas as animal feed.

Lectins

Lectins, or carbohydrate-binding proteins, occur throughout the plant kingdom, and in many species are accumulated in plant tissues as defensive proteins, being particularly abundant in seeds and other storage tissues, where they can account for up to 1% or more of total protein (reviewed by Peumans and van Damme and van Damme et al. [166, 167]). They are multimeric proteins containing polypeptides which range from 10 to 35 kDa in size. The insecticidal activity of lectins was first observed in assays with larvae of coleopteran species (e.g., LE QA done [168, 169]), where retardation of development, and in some cases, mortality, was observed when lectins were incorporated into diets at 1–5% of total protein. Lectins have only relatively low antimetabolic effects on lepidopteran larvae when fed in diet [170], possibly as a result of high gut pH inactivating the carbohydrate-binding activity. The mechanism of toxicity of these proteins remains obscure, but is dependent on carbohydrate binding.

Although transgenic tobacco and potato plants expressing lectins from garden pea [171] and snowdrop [172] have been produced by standard transformation techniques, and have been shown to confer partial resistance to lepidopteran larvae (>50% reductions in plant damage, with increased larval mortality and decreased growth), the availability of better insecticidal genes specific for these pests has directed this approach toward different targets. Homopteran plant pests, which are not affected by known *Bt* toxins, were shown to be susceptible to lectin toxicity when the proteins were delivered via artificial diet [173]. Susceptibility varied between species, and between lectins, but LC_{50} values as low as 6 μM have been estimated (for snowdrop lectin fed to rice brown planthopper (*Nilaparvata lugens*); [174]). Expression of the mannose-specific snowdrop lectin (*Galanthus nivalis* agglutinin; GNA) in transgenic rice plants was carried out, using both a phloem-specific (rice sucrose synthase) and a constitutive (maize ubiquitin-1) promoter [175]. The resulting plants were partially resistant to rice brown planthopper, with reductions of up to 50% in survival of immature insects to adulthood, and reduced development and fertility of survivors. Results were confirmed by independent transformations of indica rice varieties [176]. GNA-expressing rice was also resistant to other homopteran plant pests, such as green leafhopper (*Nephotettix virescens*; [177]) and whitebacked planthopper (*Sogatella furcifera*; [178]). Plants expressing both GNA and Cry1Ac were protected against both brown planthopper and striped stem borers (*C. suppressalis*), but no synergistic effects between the two insecticidal proteins was observed [179]. Further progress on this research has been limited, due to concerns about possible adverse consequences to higher animals of ingesting snowdrop lectin. While earlier data must be regarded as unreliable, a recent study found that no adverse effects of consumption of transgenic rice expressing GNA by rats, although significant differences in some parameters to a control group were observed [180]. GNA expression has also been engineered into potato [181] and maize [182], to give partial resistance to peach–potato aphid (*Myzus persicae*) and corn leaf aphid (*Rhopalosiphum maidis*), respectively. However, these insects are insensitive to lectin toxicity, and only marginal effects on fecundity were observed.

Introduction of foreign lectin genes into plants has become established as a potential method for engineering insect resistance, although with the lectins tested at best only partial protection against homopteran pests is conferred, and some species are relatively insensitive to the effects of lectins. As is the case with PIs, it is likely that plant pests are preadapted to the presence of lectins as defensive compounds, and are able to tolerate the toxic effects to varying degrees, although responses induced in insects by ingested lectins have not been characterized. Attempts have been made to select lectins which are the most effective toxins against target insect pests; a mannose-specific lectin expressed specifically in garlic leaves (ASA-L) was observed to show a high level of toxicity toward homopteran pests [183]. A gene encoding this lectin has been engineered into a variety of transgenic plant species, including tobacco [184] and Indian mustard [185], in both cases producing partial resistance to aphid species, with reduced survival and fecundity. Expression of this lectin in transgenic rice using constitutive [186] or phloem-specific promoters [187] gave protection against homopteran pests comparable to, or slightly better than, earlier transformations using gene constructs encoding GNA. The transgenic rice plants expressing ASA-L were shown to decrease transmission of Rice Tungro Virus by its insect vector (green leafhopper), presumably by causing decreased feeding by the pest [188].

Despite these encouraging results, widescale adoption of transgenic crops expressing lectins will probably not occur unless a major commercial company is able to gain exclusive marketing rights, and invests in pushing the transgenic varieties through the regulatory process. This is unlikely to happen, as the technology is not readily protectable by patenting.

Oxidative Enzymes

Induction of polyphenol oxidase (PPO) synthesis is one of the end-results of the plant wounding response [189], and it would seem reasonable to suppose that increased levels of this enzyme would lead to enhanced resistance to insect attack. PPO activity leads to tissue browning, which has been correlated with enhanced insect resistance. The oxidative cross-linking of tannins to proteins catalyzed by PPO decreases protein digestibility, and limits nitrogen availability [190]. However, there is little or no evidence that PPO levels are correlated with insect resistance (e.g., [191]). High-level, constitutive over expression of a poplar PPO gene in transgenic poplar seedlings led to levels of PPO up to 50x higher than normal in plant tissues [192], but these plants had only marginal effects on larvae of the lepidopteran insect pest forest tent caterpillar (*Malacosoma disstria*). No feeding deterrence was observed, and there was no effect on larval growth or survival except under conditions where larval survival was poor on controls. PPO activity was detected in insect gut and frass, so the negative results were not due to enzyme inactivation. The conclusion that herbivorous insects are preadapted to be able to deal with PPO activity, as a result of exposure to the wounding response on an evolutionary timescale (in a similar manner to preadaptation to PIs – see above) is difficult to avoid.

Peroxidase activity is also induced when plants are stressed, or attacked by pathogens, as part of a lignification response, and several attempts have been made to over-express peroxidases in transgenic plants to enhance insect resistance, despite a lack of clear-cut evidence that peroxidase activity in plant tissues is toxic to insect herbivores. Initial results using tobacco as the host plant, with over-expression of tobacco anionic peroxidase, showed only marginal effects [193], although limited broad-range protection against a variety of pests was observed in the field [194]. The limited protection afforded by this technique argues against further development.

Other Plant Proteins

Ribosome inactivating proteins (RIPs) and chitinases have also been viewed as defensive proteins in plants, although it is not clear that they are part of defense against insect herbivores. Both types of proteins have been expressed in transgenic plants, with variable results in conferring insect resistance. Expression of a maize RIP in transgenic tobacco resulted in very low levels of protection against corn earworm (*H. zea*), which were barely statistically significant [195]. Plant chitinases in general show low toxicity toward insects, but a poplar chitinase, designated WIN6, was selected on the basis that its expression was induced by insect attack. Expression of WIN6 in trangenic tomato plants

led to partial protection against larvae of Colorado potato beetle, with retardation of larval development observed [196]. Expression of the chitin-degrading enzyme *N*-acetylhexosaminidase from *Arabidopsis* in various transgenic plant tissues also gave some protective effects against lepidopteran larvae [197], but it is difficult to see what advantages over other strategies this approach could give. Orally ingested insect chitinases are strongly toxic to lepidopteran larvae (e.g., [198]). However, expression in transgenic plants gave only partial protection against insect herbivores [199], or, in one case, increased susceptibility to attack [200]. Expression of chitinase A from baculovirus AcMNPV in transgenic tobacco gave similar results, with only small effects on lepidopteran larvae and aphids [201].

Engineering Secondary Metabolism for Plant Defense

Compounds synthesized as the end-products of secondary metabolism play major roles in both constitutive and induced defense against insect herbivores in many plant species (reviewed by Wittstock and Gershenon [202]). The idea that these compounds could be used as insecticides has been a part of agriculture for thousands of years, and has been exploited successfully by synthetic chemistry in the production of classes of insecticides such as pyrethroids, based on terpenoid esters produced by flowers of pyrethrums (Chrysanthemums). Although the concept of synthesizing a foreign, insecticidal secondary metabolite in a transgenic plant developed concurrently with plant transformation technology, the biosynthesis of most secondary compounds was poorly understood, and the necessity of cloning and introducing a series of genes expressing biosynthetic enzymes to produce a secondary metabolite was considered beyond the techniques available at the time. Anticipation of problems in ensuring controlled co-expression of a series of biosynthetic genes has proved to be over-pessimistic, and plants containing multiple expressing transgenes have been produced without difficulty.

The explosion of knowledge brought about by large-scale cDNA sequencing programs and the *Arabidopsis* genome program has resulted in a much better understanding of secondary metabolism, with many biosynthetic pathways now reasonably well understood, and clones encoding biosynthetic enzymes available. The first successful demonstration that a foreign secondary compound could confer insect resistance in a transgenic plant [203] exploited a biosynthetic pathway for cyanogenic glycosides. The cereal *Sorghum bicolor* produces a cyanogenic glycoside, dhurrin, by a biosynthetic pathway starting from the amino acid tyrosine, a product of primary metabolism. Two oxidation reactions catalyzed by cytochrome P450 oxidases generate *p*-hydroxymandelonitrile, which is then glycosylated by a UDP-glycosyltransferase to form dhurrin. The three sorghum enzymes responsible were cloned and assembled into expression constructs using constitutive (CaMV 35S) promoters [204], and *Arabidopsis* plants were successively transformed with a construct containing both P450 oxidase sequences, and the glycosyl transferase sequence. All the enzymes were localized correctly (to endoplasmic reticulum membranes) and functioned properly. Surprisingly, little disruption to endogenous metabolism was observed in the transgenic plants expressing medium levels of dhurrin, and accumulation of pathway intermediates was not observed. The implication is that the plastic nature of plant metabolism can accommodate and regulate activity in new biosynthetic pathways that are introduced. The resulting plants included individuals producing levels of dhurrin similar to sorghum plants in leaf tissue (up to 4 mg/g fresh weight) and produced hydrogen cyanide on tissue damage (due to the hydrolysis of dhurrin by an endogenous *Arabidopsis* enzyme). The dhurrin-expressing plants showed enhanced resistance to attack by the flea beetle *Phyllotreta nemorum*, a specialist feeder on crucifers; adult beetles avoided feeding on dhurrin-expressing leaves when offered a choice, and larvae under no-choice conditions either failed to initiate feeding, or on initiating feeding showed a significant level of mortality. These initial results clearly imply that production of high levels of dhurrin in transgenic *Arabidopsis* caused phenotypic abnormalities, but subsequent refining of the technology allowed accumulation of dhurrin at up to 4% dry weight in *Arabidopsis* tissues without deleterious effects on plant growth [205]; expression levels of the UPD-glycosyl transferase must be high enough to prevent accumulation of the *p*-hydroxymandelonitrile intermediate.

Although these results represent science of the highest quality, this method is of marginal usefulness for crop protection as it stands, due to the dhurrin end product being toxic to higher organisms, due to the production of hydrogen cyanide when it is hydrolyzed. Worse, many insect herbivores, particularly those which have a polyphagous feeding habit, can detoxify cyanide [206]. However, the feasibility of engineering secondary metabolism in crop plants has now been established. Expression of the cassava cyanogenic glycosides, linamarin and lotaustralin (derived from valine and isoleucine respectively), has also been achieved in *Arabidopsis* [207], and grape vine root cultures have been engineered to produce dhurrin [208], although in this case no protection against root aphids was observed. Other types of secondary metabolites have also been exploited; production of the alkaloid caffeine from its precursor xanthosine in tobacco was achieved by the introduction of three genes encoding *N*-methyl transferases [209]. The resulting plants contained up to 5 μg/g fresh weight caffeine in leaves, and showed a strong feeding deterrent effect toward a generalist lepidopteran herbivore, *Spodoptera litura*. An alternative approach to modifying secondary metabolism was taken by [210], who introduced a gene encoding β-glucosidase from *Aspergillus niger* into tobacco plants, and demonstrated that transgenic plants expressing the enzyme had insecticidal activity toward whiteflies (*Bemisia* spp.) and dipterans (flies), putatively due to hydrolysis of unidentified glycosides in the plant (although the greater density of secretory trichomes observed in transgenic plants may also have been significant). Further developments in this area can be expected.

Besides engineering, secondary metabolism to produce defensive compounds normally present in other plant species, the biosynthetic capacity of plants can be used to produce a variety of volatile secondary compounds used for communication. Better understanding of the terpenoid biosynthesis pathways has led to the production of a number of transgenic plants with altered volatile composition (reviewed by Aharoni et al. [211]). Suppression of expression of a cytochrome P450 oxidase gene expressed in trichomes by RNAi led to transgenic tobacco plants which deterred aphid colonization [212], due to the final step in production of the diterpenoid cembratriene-diol being blocked, resulting in accumulation of the precursor, cembratriene-ol. These compounds are both volatile and components of trichome secretions. Transgenic *Arabidopsis* plants constitutively over-expressing a dual linalool/nerolidol synthase in plastids produced significant amounts of linalool, both as a free alcohol (volatile) and as glycosylated derivatives, and were repellent to aphids (*M. persicae*) when tested in a choice experiment [213]. Modifications to isoprenoid synthesis in *Arabidopsis* have also been shown to attract predatory mites, which could protect plants by destroying pests [214]. This strategy of attracting natural enemies to pests has also been exemplified by transforming *Arabidopsis* with the maize terpene synthase gene TPS10, which is responsible for producing sesquiterpene volatiles emitted by maize. The resulting plants emitted the volatiles normally produced in maize and attracted parasitoid wasps which attack maize pests [215]. A different approach to utilizing terpene production in transgenic plants exploits the activity of the sesquiterpene (E)-β-farnesene as an alarm pheromone in aphids, which causes cessation of feeding and avoidance, as well as acting as an attractant for aphid predators and parasitoids [216]. *Arabidopsis* was transformed with an (E)-β-farnesene synthase gene from mint, under control of a constitutive promoter (CaMV 35S); resulting plants produced (E)-β-farnesene as a volatile. The transgenic plants showed significant levels of aphid deterrence in choice experiments, and were attractive to the aphid parasitoid *Diaeretiella rapae*. Experiments which engineer the volatiles emitted by plants are an exciting area of research at present, which has established the role that volatiles emitted by plants play in the interactions between plants, herbivores, and natural enemies at the tritrophic level. This technology has yet to show that it is a practical method for crop protection in the field, but practical applications look likely to follow.

Some Novel Approaches

Many other approaches to engineering insect resistance in transgenic plants have been proposed, and progressed to varying degrees. The following section gives an overview of some of the most promising of these approaches, which have been taken forward to the stage of demonstrating feasibility by producing insect-resistant plants.

Of necessity, many other interesting ideas have had to be omitted, such as transformation of plants with transcription factors which alter gene expression [217, 218], or the use of transgenic plants expressing potentially toxic proteins from insects [219] or insect peptide hormones [220]. Despite the lack of commercial deployment of any of the insect-resistant transgenic plant other than those expressing proteins derived from *Bt*, this field of research is active and new approaches will continue to be put forward and evaluated.

Photorhabdus luminescens Insecticidal Proteins

Photorhabdus luminescens is an enterobacterial symbiont of entomophagous (insecticidal) nematodes of *Heterorhabditis* species, used for small-scale biological control of insect pests. The bacteria are present in the nematode gut, and when nematodes enter an insect host, bacterial cells are released into the insect circulatory system. The bacterial cells release toxins which cause cell death, leading to a lethal septicemia, providing a substrate for both bacteria and nematodes to grow on [221, 222]. The toxins are present as high-molecular-weight (M_r approx. 10^6) complexes, which are toxic when injected or fed to insects from four major orders of agricultural pests. The complex has been separated into four components, encoded by genetic loci *tca*, *tcb*, *tcc*, and *tcd*; the products of *tca* and *tcd* are toxic individually when fed to lepidopteran larvae. The mechanism of action of the toxins remains unresolved. Subsequent investigation has shown that *Photorhabdus* contains a large number of potentially insecticidal components, some of which are only toxic by injection, whereas others are orally toxic (reviewed by ffrench-Constant [223]); a variety of mechanisms of toxicity, including promotion of apoptosis, seems to be exploited by the bacterium. This presence of a reservoir of redundant insecticidal activities, reminiscent of the situation in *Bacillus thuringiensis*, led to *Photorhabdus* being put forward as a successor to *Bt* as a source of insecticidal genes for expression in transgenic plants.

In order to be able to exploit insecticidal genes, investigators have sought to isolate single toxic proteins from *Photorhabdus*. Two proteins, designated toxin A and toxin B, were isolated from culture supernatant and shown to be orally toxic [224]. They exist as high-molecular-weight complexes (approx. 860 kDa) in solution, and each consist of two polypeptides, 201 and 63 kDa molecular weight. The mature polypeptides are produced from single precursor protoxin polypeptides of 283 kDa by proteolysis by endogenous bacterial proteinases. The 283 kDa protoxin A is the product of a gene designated *tcdA in Photorhabdus,* which has been cloned and assembled into expression constructs for use in transgenic plants. Expression levels of mRNA and protein were improved by adding 5′ and 3′ UTR sequences from a tobacco osmotin gene, but the coding sequence was not reengineered. Expression in transgenic *Arabidopsis* gave plants that contained intact protoxin, with a range of expression levels [225]; expression of toxin A at levels above 0.07% of total soluble protein in leaves gave almost complete protection against larvae of the lepidopteran tobacco hornworm (*M. sexta*). The toxin is not species specific, and leaf extracts were also toxic to the coleopteran corn rootworm (*Diabrotica undecimpunctata*). Commercial development of this technique is highly likely.

Entomophagous nematodes of *Steinernema* species also contain mutualistic bacteria, of *Xenorhabdus* species, which produce insecticidal toxins. These proteins could also be exploited to produce insect resistance in transgenic plants, but have not yet received as much attention as *Photorhabdus* toxins [223].

Cholesterol Oxidase

The identification of a protein from *Streptomyces* that was highly insecticidal to larvae of the coleopteran pest cotton boll weevil (*Anthonomus grandis*) resulted from a screening program assaying culture filtrates of different bacterial species [226]. The protein, which was toxic at levels comparable to a *Bt* three-domain Cry protein, was identified as a cholesterol oxidase. It was able to lyse the midgut epithelium in the insect. The mechanism of action involves the activity of the enzyme, since no activity is seen in lepidopteran larvae where the gut pH is high, and the enzyme has low activity, but may also involve effects on membrane-bound alkaline phosphatase [227]. Oxidation of membrane sterols such as cholesterol in the insect gut epithelium can destabilize membranes, leading to cell lysis as observed. However, expression of this protein in transgenic plants could prove problematic, since

it is equally capable of oxidizing sterols in plant cell membranes. The encoding gene for the cholesterol oxidase was isolated, and assembled into expression constructs containing either the complete coding sequence, the mature protein coding sequence, or the coding sequence fused to a chloroplast targeting peptide from the *Arabidopsis ribulose* bisphosphate carboxylase (RuBisCO) small subunit gene [228]. No codon optimization was carried out. Transgenic tobacco plants were produced by transformation of the nuclear genome, and all constructs were shown to result in synthesis and accumulation of active enzyme. The constructs which omitted the chloroplast targeting peptide caused protein to accumulate in the cytoplasm, and these plants were developmentally abnormal, possibly as a result of interference with plant sterol hormone signaling pathways. Plants in which the enzyme was localized in chloroplasts were phenotypically normal. Leaf tissue from all transgenic plants was toxic to boll weevil larvae when fed as a component of an artificial diet.

This work does not seem to have been progressed beyond the stage of a demonstration of concept, and no further references to it are present in the scientific literature. This gene would seem a good candidate for introduction into the chloroplast genome to engineer insect resistance, although potential effects on chloroplast membrane systems would remain a drawback.

Avidin as an Insecticidal Protein

Exploitation of the biotin-binding properties of the avian egg white protein avidin (and its bacterial functional homologue, streptavidin) in a variety of biochemical techniques has obscured its role as a defensive protein, which is toxic to bacteria. The antibacterial activity is based on its essentially irreversible binding of biotin, leading to this essential enzyme cofactor being unavailable. The insecticidal activity of avidin was recognized in 1993, when assays carried out in artificial diet showed toxicity to coleopteran and lepidopteran larvae at levels as low as 10 ppm in diet (estimated as of the order of 0.01% of total protein), although the level necessary to show toxicity was up to 100x higher for other pest species. The toxic effect was eliminated by addition of biotin to diets, suggesting that the mechanism of avidin insecticidal activity is also through biotin sequestration. Both growth reduction and mortality were observed, and the suggestion was made that gene constructs expressing avidin could provide protection against insect pests in transgenic plants [229]. Subsequent assays confirmed that susceptibility to avidin as an insecticide varies widely between different insect species, and that biotin carried over in the egg between generations had a significant effect on subsequent avidin toxicity [230].

Initial reports of expression of avidin in transgenic maize were focused on producing the protein as a high-value product [231]. An expression construct containing a codon-optimized avidin coding sequence with an N-terminally fused signal peptide from barley α-amylase, driven by the maize ubiquitin-1 promoter, resulted in expression levels of avidin of >2.0% of total protein in seed. Seed from these plants was subsequently bioassayed for resistance to larvae of three different coleopteran storage pests, including red flour beetle (*Tribolium castaneum*), with 100% mortality at avidin levels above 100 ppm of seed (approx. 0.1% of total protein). However, not all pests were as susceptible; larvae of the larger grain borer, *Prostephanus truncatus*, were effectively insensitive to avidin, whether added to artificial diet or expressed in transgenic plant material. The engineered maize was nontoxic to mice over 21 days [232]. Subsequent reports confirmed the insecticidal effects of avidin expressed in transgenic plants: these include protection of tobacco against noctuid lepidopterans [233], using vacuolar targeting sequences from potato proteinase inhibitors to direct avidin accumulation in the vacuole at levels up to 1.5% of total leaf protein [234]; protection of apple against lepidopteran pests [235]; and protection of rice against coleopteran stored grain pests, using a similar approach to that used for maize [236]. Targeting of the foreign protein to vacuolar or similar compartments is important; expression of streptavidin in tomato using plant and bacterial signal peptides and strong promoters led to developmental abnormalities in the plants, which could be corrected by topical application of biotin, suggesting that sequestration of cellular biotin is equally detrimental for plants as well as insects [237].

Despite many promising results, this technology appears to have failed to gain any acceptance for agricultural crops, as illustrated by a recent study in which seed meal from transgenic avidin-expressing maize was tested as an insecticide for topical application to stored maize [238]. Studies have shown that avidin can increase the protection afforded by *Bt* expression in transgenic plants against insect pests which have limited susceptibility to the toxin (e.g., potato expressing Cry3A; [239]), but it is clear that little further development in this area is taking place.

RNA Interference Using Double-Stranded RNA

Downregulation of gene expression by double-stranded RNA (dsRNA) corresponding to part or all of a specific gene transcript has been used as a research technique in insect genetics since 1998. The method has been based on delivery of synthetic dsRNA produced in vitro by injection into insect cells or tissues, which is clearly not practical for applications in crop protection. However, recent results have shown that dsRNA can be introduced into insects as a component of artificial diet, and is effective in downregulating genes normally expressed in gut tissue. This technique has been used to downregulate the production of a gut carboxylesterase in larvae of the lepidopteran *Epiphyas postvittana* (light brown apple moth; [240]), leading to suppression of mRNA in the insect. More significantly, two recent papers show that dsRNA can be delivered to insect pests by expression in plant material, and that this can lead to an insecticidal effect when pests are exposed to plants. Transgenic tobacco and *Arabidopsis* plant material expressing dsRNA directed against a cotton bollworm detoxification enzyme (cytochrome P450 gene CYP6AE14) for gossypol suppressed expression of the gene, and caused the insect to become more sensitive to gossypol in the diet, leading to reduced performance compared to controls [241]. A similar technique was used to suppress expression of a V-type ATPase in larvae of the coleopteran *Diabrotica virgifera virgifera* (Western corn rootworm); transgenic corn plants producing dsRNA directed against this gene showed protection against feeding damage by the insect [242]. The feasibility of using dsRNA in crop protection strategies has thus been demonstrated. This approach holds great promise for future development, as it allows a wide range of potential targets for suppression of gene expression in the insect to be exploited.

Insect-Resistant Genetically Engineered Crops and Sustainability

The success of Bt-expressing crops in the field has been a direct result of taking "sustainability" into account in their introduction, particularly with respect to managing the emergence of pest resistance to the toxins through the refuge strategy, as described earlier. Even organizations hostile to Genetic Engineering technology, such as organic growers in the USA, have reported that *Bt* cotton and corn have reduced insecticide usage significantly (by up to 0.2 kg/ha/year), showing that these crops are compatible with the goals of "sustainable" agriculture [243].

The "sustainability" of transgenic insect-resistant crops has also been examined in terms of potential effects on the wider ecosystem in which the plants are grown. Numerous studies have been carried out to effects on predators and parasites at the third trophic level, and on nontarget insects and other invertebrates. Some initial reports which did report negative effects were based on dubious assumptions, or used experimental designs which had little relevance to field conditions (e.g., the supposed threat to monarch butterflies posed by transgenic *Bt* corn; reviewed by Gatehouse et al. [244]). Nevertheless, it must be the case that if a pest population is decreased as a result of endogenous resistance in crops, then there will be a "knock on" effect to the wider ecosystem, and particularly to predators and parasites of the pest species, when the resistant crop is compared to a nonresistant one that is not treated with pesticide. However, this is not a realistic comparison, since in agricultural practice a crop that does not have endogenous resistance is treated with exogenous insecticides. The use of the refuge strategy allows significant pest populations to be present, and thus can support both beneficial insects which attack the pest, and a wider ecosystem, which would be destroyed by exogenous insecticide application.

Looking to the future, wider use of insect-resistant transgenic crops could contribute positively to "sustainability" in agriculture in general, by further decreasing

insecticide usage and thereby decreasing energy inputs. However, the "sustainability" of the insect-resistant crops themselves is going to come under increasing pressure, as less controlled deployment of insect-resistant plants evades the present compulsory use of the refuge strategy, and use of crop varieties with multiple *Bt* toxins renders the refuge strategy apparently less necessary to prevent pest resistance to *Bt* toxins developing. Field resistance to *Bt* crops has been observed recently (reviewed by Tabashnik et al. [245]), but is manageable using existing practices, or modifications of them. The sustainability of relying on one mechanism of crop protection can be questioned, especially as plants in general have evolved mixed defense strategies [246]. In the longer term, a wider range of strategies for producing insect-resistant plants is going to be necessary, not only to deal with the potential for nonspecific resistance to *Bt* toxins, but to extend the range of crop pests that can be targeted, and further reduce the application of pesticides.

Future Directions

After 20 years, insect-resistant transgenic crops have been a greater success in some ways than the early experiments suggested, but have failed to meet all the hopes that were initially raised. The success is self-evident when the widescale adoption of the technology in certain crops such as cotton and maize is considered, and documented evidence of reductions in damage to human health and the environment as a result of decreases in the use of exogenously applied pesticides. The failure does not lie in any technical shortcomings in the science, although improvements and new strategies are always possible; it lies in a failure to disseminate the technology as widely as should have been the case, so that it remains largely in the hands of commercial organizations, and is limited to the major crops. Is it an unrealistic hope to anticipate that after another 20 years, amateur gardeners in developed countries will be able to choose to buy seed to grow genetically engineered cabbages, which will be resistant to cabbage white butterfly larvae, in their allotments and gardens? Or that rural farmers in developing countries will have free access to engineered rice varieties, suitable for their growth conditions, that are resistant to pests such as stemborers? Both these aims have been scientifically achievable for at least the last 10 years, and it is surely about time that a more rational approach, which cuts through both the largely futile debate about the rights and wrongs of plant genetic engineering, and the protectionism of agrochemical companies, was taken to address the looming problem of producing enough crops to meet humanity's needs.

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Genotype by Environment Interaction and Adaptation

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Article Outline

Glossary

GE *Genotype by environment interaction* is differential genotypic expression across environments that may cause that a genotype selected among the best in one location to perform poorly in another. GE weakens association between phenotype and genotype, reducing genetic progress in breeding programs. In statistical terms, GE describes a situation in which the simultaneous effect of two classification variables (genotype and environment) on a continuous dependent third one, such as yield, does not follow an additive model.

MET A *multi-environment trial* is a series of trials sampling the target environmental range in which a particular set of genotypes is evaluated.

QTL A *quantitative trait locus* is a region in the genome associated with a particular quantitative phenotypic trait, such as crop yield, resource-use-efficiency, phenology, or height. QTL analysis is a statistical method that links phenotypic data (specific trait measurements on a series of individuals) and genotypic data (usually in the form of molecular markers taken on the same individual) in order to explain the genetic basis of complex traits. QTL number and the variation they explain on the phenotypic trait give clues about the genetic control of that trait, for example, if plant height is controlled by many genes of small effect, or by a few genes of large effect.

QTLxE QTL by environment interaction is differential QTL effect across environments that may cause that a favorable QTL in one environment may become irrelevant, or even unfavorable, in another.

Specific and wide adaptation A genotype is considered stable if it yields well relative to the productive potential of the environments in which is grown. If such concept of stability is shown for a wide agroecological array of environments, a genotype is considered to have general, wide, or broad adaptation. If stability is confined to a limited range, a genotype is said to have specific or narrow adaptation.

Definition

One of the first decisions farmers have to take is the selection of the variety to be grown in their fields based on expectation of economic returns, generally, in the form of the highest attainable yield. This is a critical choice that strongly determines the sustainability of the agricultural system. However, this is by no means trivial as it is very hard to identify the "best" variety across a diverse set of environments subjected to complex biotic and abiotic factors and interactions generally causing significant changes in varietal rank. Therefore, a major objective in plant breeding programs is to determine the potential adaptation of advanced breeding lines across a range of agroecological conditions. William S. Gosset (who signed as "Student [1]" in a landmark publication introducing the t distribution) wrote at the onset of modern breeding that the ultimate purpose of field experimentation was to determine what varieties pay farmers best. He thought that the design of experiments should aim, not only at determining the average yield, but also at identifying varieties whose yield, being within those of high average value, were relatively less responsive to variation in soil and climate.

Breeding programs normally aim to release cultivars to be successfully grown over a rather large cropping area, varying in soil quality attributes and in

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average climate, and across several growing seasons, with interannual variations in climatic conditions. The target environment is defined as the set of soil × climatic conditions in which the released cultivars will be grown and to which the cultivars must be adapted. Therefore, a key step in applied plant breeding is the identification of advanced genotypes broadly or narrowly adapted across a wide range of target environments. Breeders focus in the first segregating generations on direct phenotypic selection of highly heritable traits, such as plant architecture and phenology to concentrate in later stages on complex quantitative traits like yield and end-use quality. Marker-assisted selection aims at complementing this phenotypic selection with direct marker screening for, mostly, oligogenic-controlled traits. The traditional approach to estimate the genotypic value in the context of breeding, varietal registration, and recommendation is deployment of extensive field evaluation schemes in a series of sites in which the assessed genotypes could be potentially grown. These collections of trials are generally denominated multi-environment trials (METs) in which a set of genotypes is evaluated in a series of trials that sample the target environmental range. Data from METs are typically summarized in the form of genotype by environment tables of means. Simple inspection of such tables of means will often reveal the presence of genotype by environment interaction (GE) or differences in performance of genotypes that are trial dependent. They also allow for the identification of those genotypes that are partially or generally adapted to the environmental range, showing specific or narrow versus general or wide adaptation, respectively.

The traditional outcome of METs is the identification of "which" cultivar and "where" has performed well. These studies are empirical, based on simple statistical characterizations of genotypic responses across environments and do not provide any physiological insight into the basis of the genotypic response to environmental changes. However, as one wants to move forward toward a *predictive* breeding scenario, the challenge beyond "which" and "where" is "why" narrow or wide adaptation happens, in terms of a thorough understanding of both the environment, the physiological behavior of the different cultivars and, eventually, of the genes responsible for adaptation. Identifying the "why" is not only a matter of satisfying curiosity: It would potentially allow more precise breeding through the direct manipulation of the genes responsible for the different adaptation patterns.

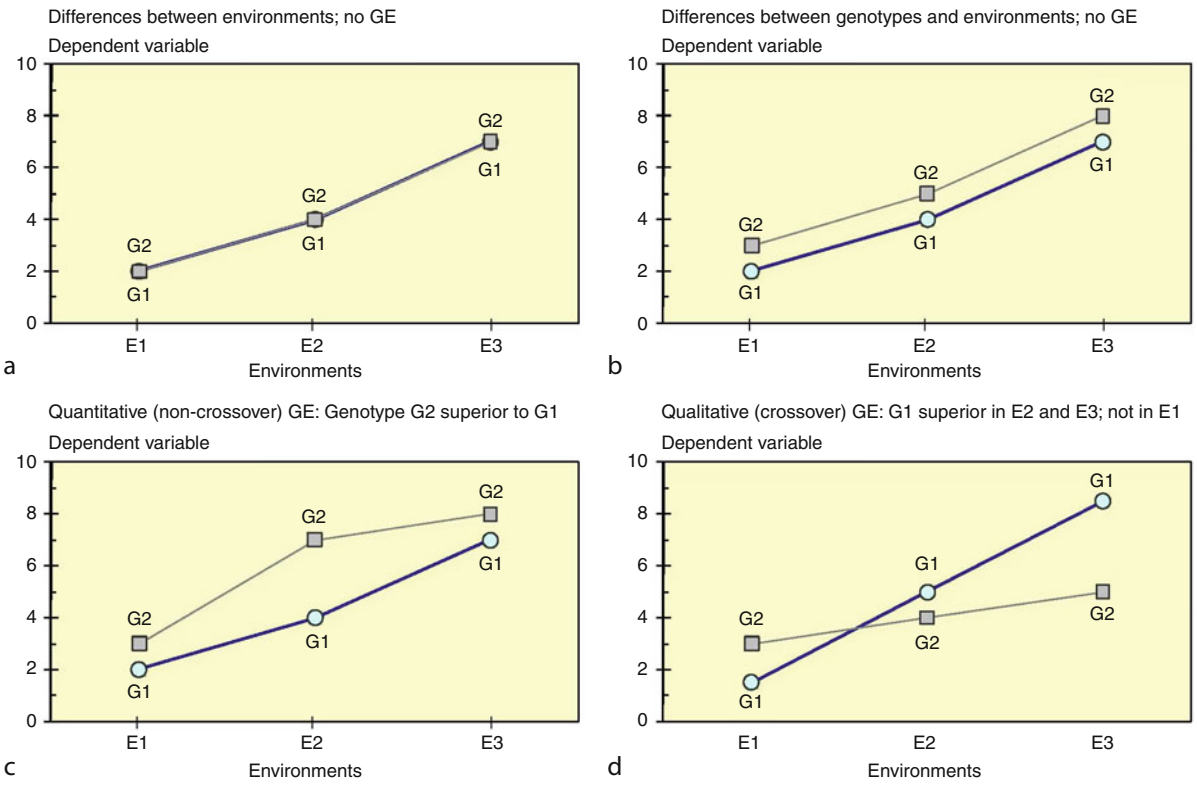
Introduction

Statistical analyses that detect and describe GE have been comprehensively reviewed [2–14]. Means across environments in METs are only adequate estimates of varietal performance in the absence of GE. When GE is significant, average values across environments may hide subsets of environments where genotypes differ markedly in relative performance.

As for any other statistical two-factor model, there are different types of interactions which originate from departure from additivity. In Fig. 1, the average for each of two genotypes, G1 and G2, for the dependent variable of interest, for example yield, is shown for three environments. Figure 1a represents the situation in which differences were detected only between environments. Figure 1b shows an additive model in which differences for both main effects, genotypes and environments, were observed but no GE. Figure 1c shows a quantitative or non-crossover interaction; in this scenario, genotypes with superior means can be recommended for all environments. In plant breeding, the most important GE is of the crossover or qualitative type (Fig. 1d), which implies changes in the rankings of genotypes across environments). In this case, variety G2 may be recommended for environment E1 but not for E2 and E3.

When there are genotypic differences among the varieties tested and the target environments include different soils and variable climate, MET analyses more often than not detect crossover GE (only MET with limited genotypic and/or narrow environmental diversity might reveal negligible or nonsignificant interaction). Thus, identification of superior genotypes is complicated by qualitative GE and largely depends on extensive field testing conducted over years at different locations. Therefore, there is a strong need to deploy powerful statistical models for MET data taking into account GE and their breeding implications.

Crossover interactions represent a double-edged sword [10]. Whereas they make breeding, testing, selection, and varietal recommendation more difficult, if the



Genotype by Environment Interaction and Adaptation. Figure 1
Performance of two hypothetical genotypes in three environments showing: (**a**) Only environmental differences; (**b**) No GE; (**c**) Quantitative, non-crossover, GE; (**d**) Qualitative, crossover, GE

underlying ecophysiological grounds of GE are known, identification of genotypes better adapted to certain specific niche conditions, allowing for increased genetic gains, is possible. If the traits conferring adaptation to these specific environments and/or the genes that control them are revealed, direct implementation in breeding may be feasible either by choosing parents for a new cross possessing the adaptive attributes or by directly selecting for the presence of such attributes in the progenies (through direct measurement of the attributes or through genotypic selection, see below).

METs are often carried out over a number of sites and years that are considered to be representative of the target environments. Standard analyses of variance partition the GE term into genotype by locations (GL), genotype by years (GY), and genotype by locations by years (GLY) interactions. The relative size of these terms allow for a statistical assessment of the spatial and temporal components of adaptation. If GL dominates over the other components, then specific adaptation is exploitable by identifying subsets of homogeneous locations for variety release and recommendation. Where GY and GLY terms dominate, as most often happens, no simplification involving spatial subdivision of growing regions is possible. In this context, specific recommendations may be only possible after counting with robust models trustworthily predicting the main climatic conditions of the growing season in advance to sowing.

Recent efforts have searched for the genetic factors underlying GE and, thus, to describe adaptation patterns. Quantitative trait loci (QTLs) responsible for individual complex traits (see, e.g., [15]), such as yield and adaptation have been reported in several populations for most crop species. QTL related to adaptation show different effects in different environments. The magnitude of individual QTL effects (expressed as the amount of GE variation explained

by a particular QTL) varied among populations and across environments. Therefore, implementation of marker-assisted selection strategies for these QTLs in applied breeding programs remains a challenge. Modern GE studies have introduced external environmental, physiological, and/or genetic information to develop statistical models whose parameters relate better to physiological knowledge [16, 17], and therefore offer better possibilities for implementation of QTL selection methodologies in breeding programs.

Breeding Implications

Historically most of the genetic progress in the last decades at the global level, particularly in cereals, has been attained through increases of yield potential and disease resistance. Genetic gains in yield under non-limited growing conditions, i.e., improving yield potential, have often brought about parallel gains in yield under a wide range of more realistic, largely stressful, growing conditions [18–20]; because physiological traits behind improved yield potential may often be constitutive and provide yield advantage over a range of conditions [21]. Thus, improving simultaneously for yield potential (which is directly linked to both attainable and on-farm yields; [22]) and for disease resistance has conferred not only clear progress under high-yielding conditions but also wide adaptation.

Thus, it is critical to further improve yield potential [23]. Lessons from the past allow to optimistically trusting that relatively simple traits might be found that affect yield potential and wide adaptation simultaneously (e.g., [24]). For instance, the incorporation of simple key traits such as reduced height might have such a great impact that may be the basis of a Green Revolution due to its capacity of increasing yield both under potential and most non-potential conditions. Genetically reducing the capacity of the stems to grow through introgression of semidwarfing genes determined firstly an increased partitioning of biomass accumulated during stem elongation to the growing spikes [25, 26]; then the additional availability of resources in the growing spikes allowed floret development to proceed normally in more floret primordia consequently increasing the number of grains [27] and therefore parallel improvements in yield, as cereals are most frequently sink-limited during grain filling even under nonoptimal environments [28, 29]. However, as further reducing height would not keep improving yields [30], it is critical identifying alternative traits that being rather simple were still putatively related to yield across a wide range of conditions. Difficulties in identifying such traits is reflected in the fact that despite continuous breeding efforts in the last decade, current genetic progress in yield potential fall short of both those attained before (see [31] and references therein) and that required to match expected increases in demand [23]. Future improvements in yield potential would largely depend upon the identification of alternative traits that being relatively simple are putatively related to yield in a wide range of conditions representing the target environments of the breeding program. In this context, a thorough examination of GE will be critical both for identifying traits in a top-down approach dissecting yield into physiologically sound traits across conditions representing the target environments, and for determining the stability of the relationship between the identified trait(s) and yield.

In an even more general context, GE has important implications in applied breeding programs [5]. Based on the magnitude and nature of GE, breeders have to decide whether to aim for wide or for specific adaptation. This decision determines the choice of locations for selection, the allocation of limited resources in advanced line testing, and the assessment of the potential trade-off between empirical, molecular, and physiological screening of parents and advanced lines. Related to wide adaptation is the question of breeding sites: Can selection under optimum high-input environments identify genotypes adapted to more stressed environments? Salvatore Ceccarelli and Stefania Grando at ICARDA have produced a significant number of contributions on the issue of wide versus specific adaptation in barley (see [32] and their own references therein for a review). They have strongly advocated the exploitation of specific adaptation for optimum use of resources particularly in marginal environments, arguing that selection for high yield potential has not increased yield under low-input conditions. However, success of the CIMMYT wheat program aiming at wide adaptation is based on a completely different approach. Rather than focusing on any specific environmental

conditions, continuous selection cycles, referred to as shuttle breeding, are carried out in alternative and extremely diverse high yield potential environments differing in altitude, latitude, photoperiod, temperature, rainfall, soil type, and disease spectrum. As a result, CIMMYT wheat genotypes have shown high yield potential and wide adaptation across large geographical regions, perhaps with the exception of very marginal; in fact, poor adaptation of CIMMYT genotypes to specific environments often reflected susceptibility to specific plant diseases.

Field experimentation aims at covering a representative sample of environmental variation. However, the need for adequate resource allocation raises the question of whether multilocation testing in a limited number of years can adequately sample the array of environmental conditions where a variety can be grown. If the MET analysis of variance identifies GY as the most significant term, testing for many crop cycles should be preferred. However, this is not suitable given the increasing pressure to develop new cultivars. Therefore, breeders often substitute temporal for spatial environmental variation, assuming that GL is similar in nature to GY and that GLY is absent. Resource allocation for varietal experimentation schemes depends on the relative magnitude of the variance components for the genotype and GE interaction terms. Given the small number of years available for testing, and the frequently dominant effect of GY and GLY interactions, there is little point in a very extensive series of trials in a given year with a high proportion of genotypes retained throughout. Integrated mixed model analyses for the selected genotypes across the breeding stages can counterweigh for the limited number of years in the later stages of field testing.

A series of papers have suggested the use of reference and probe genotypes to characterize environmental variation and assess GE repeatability [33]. By defining a common reference set of genotypes consistently grown across locations and years, a breeder could define a long-term target environment and weight results from each location in a given year in accordance with its across-year representativeness. Probe genotypes with differential response to known biotic and abiotic conditions could also be used to characterize environments. However, practical application of these two principles is not common. Genetic gains for unidentified biotic and abiotic stresses by direct selection on extensive MET are possible. A more sound approach could be the growing of genotypes in a few key environments with well-characterized levels of the target stress. Manipulation of the breeding environment and selection of key parents for crossing should result in improved genetic gains. However, this second approach requires a clear understanding of the major stress as well as the facilities to reproduce it.

A germplasm strategy is also needed for breeding for wide and specific adaptation. For most crops, there is an important gap between elite and unimproved gene pools as most breeders focus on germplasm reflecting decades of intensive crossing, selection, and recombination [34]. However as genetic gains attained by conventional breeding decrease, more emphasis should be given to the use of new genetic variability both through pre-breeding or through construction of new parent for crosses, incorporating desired traits from local land races and related wild species, or from other unrelated organism through transgenesis.

The first studies on GE were based on standard variety trials across a series of environments. That allowed identification of the wide or narrow adaptation of the checked cultivars, but little could be said on the genetic basis of adaptation. Extensive field testing of biparental crosses (e.g., [35]), either in the form of doubled haploids, or recombinant inbred lines populations, allows for the assessment of the genetic control of plant adaptation based on standard linkage and QTL analyses, but their use is limited by the level of polymorphisms between parents. In contrast, diverse genotypic panels accumulating multiple recombination events provide ample genetic variation for association studies. However, their main limitation is the high incidence of false-positive associations due to the difficulty to distinguish between true and pseudo linkage between molecular markers and traits of interest, due to population substructure and correlated selection [36]. More recently, other more complex crossing systems have been proposed to exploit the advantages of both linkage analysis and association mapping. This is the case, for example, of the so-called MAGIC (multiparent advanced generation intercross) [37], the nested association mapping (NAM) design based on a huge set of recombinant inbred lines derived from

a large number of founder genotypes [38, 39], and AMPRIL (a multiparent recombinant inbred line population) [40].

The use of physiological criteria in analytical breeding is critical for success [41–44]. Breeders develop a deep knowledge of their target environments and of the agroecological adaptation of their genetic materials. However, whereas intensive work is continuously been carried out by crop physiologists in the area of yield potential and adaptation, not many breeders regularly incorporate new physiological criteria in their mainstream-breeding program. In any case, physiological assessment of adaptation is needed to complement breeders' impressions particularly in the first and last stages of a breeding program: selection of parents and assessment of adaptation of new advanced lines. Similarly, despite exciting progress in molecular marker-assisted selection, applied breeding still depends heavily on direct phenotypic selection of advanced genotypes.

In the rest of this entry, two different aspects will be presented: First, an example of the physiological implications of GE through the study of a trait, time to flowering, that has a clear effect on adaptation; second, a series of increasingly complex statistical models to characterize genotypic adaptation, to identify genotypes showing wide or specific adaptation and to dissect the genetic complexity behind this integrative trait. Although these sections may look quite disconnected, a thorough knowledge of crop physiology and/or their genetic control could allow construction of more powerful integrated statistical models incorporating as genetic covariables this information in order to improve the understanding of the nature of GE. Conversely, the statistical models can identify certain genotypes which, if well characterized, could allow for empirical identification of key adaptative traits.

Traits Determining Adaptation

The number of physiological traits with a potential effect in determining yield and adaptation is extraordinarily large. In an excellent *Crop Physiology* manual recently edited by Sadras and Calderini [45], many traits are reviewed and organized according to different criteria from capture and efficiency in the use of resources to crop development and plant architecture. Many trade-off exists between traits that, if ignored, will slow down genetic progress for both potential and actual farmer yields. Araus et al. [43] have also reviewed a number of potentially useful physiological criteria for breeding, particularly, in the framework of C3 cereals under Mediterranean conditions. Crop physiology as a whole is beyond the objectives of this entry. Therefore, the focus is on the single most important crop trait determining plant adaptation, time to flowering, as an example of a key trait to describe the underlying mechanisms and implications for GE.

Time to Flowering

Crop phenology – life cycle as influenced by seasonal variations in climate – has been widely recognized as the most important single factor determining adaptation and thereby crop performance. In determinate species, it allows for matching crop development with availability of resources, avoiding abiotic stresses due to climatic conditions such as late spring frosts and terminal drought. To maximize attainable yield, the most "critical phases" for yield determination have to be match with the most favorable (or least unfavorable) growing conditions. In some cases (Northern Hemisphere), the obvious way to achieve this is sowing cold-tolerant genotypes early enough to have full growth in early spring, but in the warmer Southern Hemisphere similar maximum yields can be achieved sowing in winter with significantly shorter phases, provided the critical phases are ideally timed [46–49]. Crop phenology is, thus, not only a key adaptative trait, but it may also affect yield potential, since different structures are produced throughout the crop cycle, and some of them may be more important than others in determining yield potential [50]. If the pattern of water deficit in the target region is relatively predictable, manipulation of genes responsible for crop phenology is the most sustainable approach to increase attainable yield and plant adaptation.

The importance of flowering time has been shown, for example, with the fast and diverse shifts in heading time, or in vernalization and photoperiod responses, due to natural selection: When the same bulk population is grown under contrasting environments [51]; when comparing different sowing dates [52]; when

studying the contrasting developmental patterns of genotypes adapted to particular regions [53–55]; or in retrospective studies showing changes in heading date over time due to breeding, particularly in areas where the crop was introduced more recently (e.g., bread wheat in Australia; [48]; durum wheat in certain regions of Spain; [56]). Therefore, crop phenology is an important source of GE for yield when testing genotypes from regions differing in climatic conditions [57, 58].

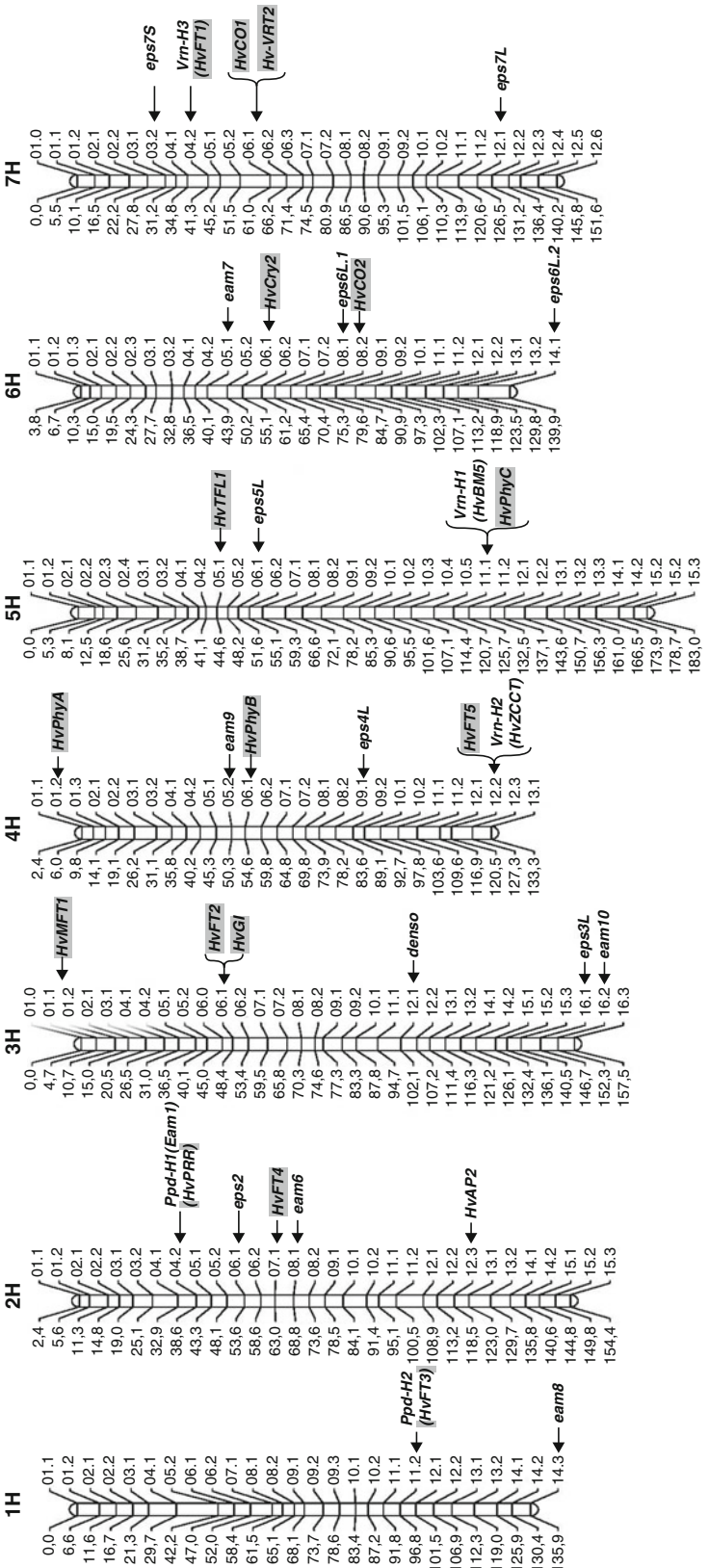
The three major factors determining flowering time are differential responses to photoperiod and vernalization and intrinsic earliness or earliness per se [50]. Further evidence from recent studies in wheat [59–61] support the idea that earliness per se genes represent basically genotypic differences in the response to non-vernalizing temperatures [62, 63]. The wide genotypic differences for these factors are considered as responsible for the spread of winter cereals, worldwide to a wide range of latitudes and altitudes [49, 64].

Genetic Factors Controlling Time to Flowering

At the gene or marker level, the importance of flowering time in crop performance is shown, for example, through the geographical distribution of alleles of major genes such as photoperiod (*Ppd*) and vernalization (*VRN*) responsive genes [49, 64, 65]. The co-location of QTLs for heading with QTLs for yield (e.g., [35, 66–69]), which may help to define an optimal window for heading or combination of alleles in the tested environments [70]. Moreover, in some of these studies, QTLs with strong effects on heading collocated with some of the QTLs for yield that exhibited strongest QTL by environment interactions [35, 69–71]. Recent studies have shown, through factorial regression, that a great part of the effect of these QTLs for heading (underlying QTLxE for yield) can be explained by the different sensitivity of the alleles to environmental conditions such as temperature during different parts of the crop cycle [11, 72].

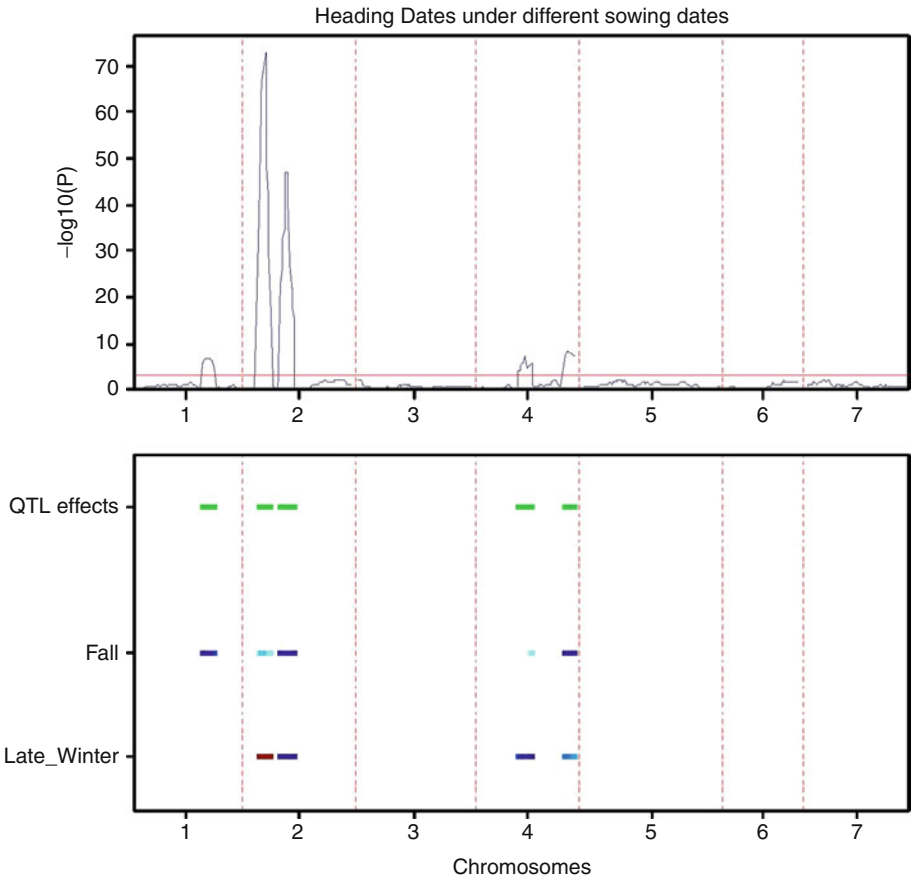
In the last decade, candidate genes have been identified for major loci controlling flowering time in barley and wheat: The photoperiod responsive gene *Ppd*-H1 in barley and its wheat homologues *Ppd*-D1, *Ppd*-B1, and *Ppd*-A1 are *PRR*-like genes [73, 74]. In both species, the photoperiod-responsive allele accelerates flowering under long-day conditions, but in barley, the greatest differences between sensitive and insensitive alleles are found under long-day conditions or high latitudes, while in wheat, under short day conditions or low latitudes [49, 64, 75, 76]. *HvFT3* is the candidate gene for another gene related to photoperiod in barley, *Ppd*-H2, whose active allele is expressed and accelerates flowering only under short photoperiod or low latitudes [75, 77]. The vernalization genes *VRN*-H1 and its homologues *VRN*-A1, *VRN*-B1, and *VRN*-D1 in wheat are MADS-box transcription factors similar to *APETALA1* in *Arabidopsis* [78–80]. *HvZCCT* and *TaZCCT* are the candidate genes for *VRN*-H2 and its wheat homologue *VRN*-Am2, respectively [81, 82]. The alleles at these loci and their interactions determine the sensitivity to vernalization (e.g., [82, 83]). Finally *VRN*-H3 and its homologues *VRN*-A3, *VRN*-B3, and *VRN*-D3 are *FT*-like genes, which also interact with PPD and VRN genes [77, 84, 85]. Other reported genes that determine differences in heading time are the "earliness per se" loci (*eps*) identified in barley by Laurie et al. [75], the series of "early maturing" (*Eam*) loci [86–89], and the gene HvAP2 [90]. However, except for the latter, no candidate genes have been found yet for them and their role is much less clear.

Figure 2 shows the location of the mentioned loci for barley, as well as for some other genes which are homologues to flowering genes in rice and *Arabidopsis* but whose effect on heading is unknown in barley. In wheat, other less characterized loci have also been identified, as the gene *Eps*-2B on 2BS [91, 92]; *Eps*-Am on 1AL sensitive to temperature [59, 60]; *VRN*-D4 close to the centromere in 5D [93], and other earliness per se genes on 5AL [94]. Additionally other loci have been found to have an effect on heading time in different regions than the loci mentioned above, although most of them with smaller effects: by the use of aneuploids in wheat [49, 95] or through QTL mapping both in barley (e.g., [35, 66–69]) and wheat (e.g., [92, 96–98]). These studies would confirm that heading time is under a strong but complex genetic control [49, 95]. Although particular VRN and PPD alleles may be more frequent in some geographical areas, variation has been found between genotypes within regions, so it is possible finding different combinations of VRN and PPD alleles in successful genotypes well adapted to particular regions, which would reinforce the idea that several



Genotype by Environment Interaction and Adaptation. Figure 2

Barley consensus function map showing the location of the vernalization, photoperiod, and earliness per se loci described in the text, as well as some other genes which are homologues to flowering genes in rice and *Arabidopsis*, whose direct effects on heading are still unknown in barley. Distances are given in Kosambi cM and linkage groups are oriented with short arms at the *top*



Genotype by Environment Interaction and Adaptation. Figure 3

Genome scan for heading date for the Steptoe × Morex doubled haploid population grown in fall and late winter sowing in Spain in 2009. *Top*: $-\log_{10}$ (*p* values) for the test on QTL+QTL.E effects are shown. The *red horizontal line* indicates the 5% genome-wide significance threshold. *Bottom*: *Upper most line* in *green* gives all genomic positions for which null hypothesis of no QTL+QTL.E is rejected. For the fall and late winter sowing environment, all positions for which there is environment-specific QTL expression are indicated with colors: *blue* showing that the allele from Steptoe delays heading, while *red*/*brown* shows that the Morex allele delays heading

other genes may be important in the control of flowering time [64]. As sensitivity to vernalization expresses at earlier stages of development than that to photoperiod, the fact that different combinations of VRN and PPD alleles may confer a similar time to heading or anthesis may also open room for fine-tuning developmental partitioning of a certain time to flowering into different lengths of vegetative and reproductive phases, which might be relevant in improving adaptation (see below).

A very simple quantitative genetic analysis of heading date (HD) for the Steptoe × Morex doubled haploid barley population [35] sown in fall and late winter in 2009 in Spain can be deduced from Fig. 3 which also illustrates alternative types of QTLxE interactions. In the top part of the figure there is, for a MET situation, a whole genome scan according to a composite interval mapping strategy [99] as implemented by Biometris, Wageningen University and Research Center, in GenStat (version 13th, [100]). All markers in the seven barley chromosomes are represented in sequential order on the X-axis. On the Y-axis is the *p* value, expressed on a minus logarithmic scale, for the successive regression models,

including not just the marker or position of interest, but additional markers that act as cofactors. With $-\log_{10}$ (p value) increasing, the evidence for a QTL at that position becomes larger. The bottom part of the figure shows firstly, in green, a one-dimensional summary of the profile in the upper panel, that is, all positions for which the joint null hypothesis of no QTL main effect and QTLxE interaction was rejected. Below the overall test for QTL effects across environments, for each individual environment, in this case defined by fall and late winter planting, an approximate test for environment-specific QTL effects is given in yellow-brown-red (QTL allele second parent increases trait) or light blue-dark blue (QTL allele first parent increases trait). Two major QTLs seem to determine heading date for the genotypes in these two trials, both on the short arm of Chromosome 2H, corresponding to two known genes, Ppd-H1 and Eam6, on Fig. 2. A very strong qualitative or crossover interaction QTLxE interaction is shown for Ppd-H1; the Morex allele (yellow-red) in the late winter sowing (under long-day photoperiod) delays heading, whereas the Steptoe allele at this locus (blue) delays heading under short days on the fall sowing. Non-crossover interaction is shown for Eam6. The presence of the Steptoe allele always delays heading, but more under fall sowing (darker blue effect) than under late winter sowing. Other minor QTLs are shown in chromosomes 1H and 4H.

Genetic Factors Controlling Duration of Subphases of Time to Flowering

The effect of these genes or QTLs may vary not only due to different conditions in temperature and photoperiod, or to epistatic interactions with other genes or QTLs, but also they may have different effects on the different phases of the crop cycle. This may be interesting for improving both adaptability and yield potential. Studying the genetic control of different pre-heading phases could bring about a better understanding of crop development patterns and more tools to fine-tuning it. For example, some adaptative characters, such as the avoidance of late frosts in spring, could be better assessed by knowing the duration of the phase from sowing to terminal spikelet rather than total time to anthesis (e.g., [101]). Moreover extending the duration of stem elongation, without modifying total time to anthesis, which is a key trait for adaptability as shown above, has been proposed as a trait to further increase yield potential [102, 103]. This has been proposed because the stem elongation phase is critical for yield determination, as the number of fertile florets at anthesis, which determines the final number of grains, is set during this phase [104, 105].

Several authors have shown that there is partially independent variability between different pre-heading phases (variability in pre-heading phases between genotypes with similar time to heading), both in wheat [106–108] and barley [109–114]. Other authors have shown that responses to vernalization, photoperiod, and temperature can each differ greatly among genotypes and between phases [50, 62, 115, 116]. In some studies using chromosome substitution lines, near isogenic lines and/or single chromosome recombinant lines, hexaploid wheat Ppd-D1 and Ppd-B1 alleles had different effects on the duration of pre-heading phases and on their response to photoperiod, although results seemed to depend on the genetic background and the environmental conditions of each experiment (see results and review by [117]). Recently Lewis et al. [61] found that alleles of a cultivar and a wild line of *Triticum monoccocum* for Eps-Am had different effects on the leaf initiation and the spikelet initiation phases (due to different sensitivity to temperature), but not on stem elongation, while they had little effect on total time to heading. On the other hand, many of the QTLs responsible for a different genetic control between pre-heading phases had little or no effect on total time to heading, so they may be more difficult to detect when assessing only heading time [111, 118]. Some of these differences in the length of pre- and post-heading phases were maintained under different conditions of photoperiod and temperature [119].

Statistical Approaches for GE Characterization

Means across environments are adequate indicators of genotypic performance only in the absence of crossover GE. When present, the use of means across environments ignores the differential reaction of genotypes to environmental changes. In an analysis of variance, introduction of the GE interaction term, $(GE)_{ij}$ for $i = 1$ to g genotypes and $j = 1$ to e environments, creates

as many parameters as there are GE combinations, making predictions of phenotypic responses for environments that were not in the set of trial environments impossible. Most approaches for the study of GE interaction and adaptation depart from ANOVA models with GE interaction terms and are therefore purely empirical descriptions of phenotypic performances of a set of genotypes across a fixed sample of environments. However, if the physiological or environmental underlying causes determining GE interaction can be determined, identification of genotypes better adapted to certain specific environmental conditions would be possible and, thus, larger genetic gains would be achievable. Furthermore, if the traits conferring adaptation and their genetic control are revealed, direct implementation in breeding may be feasible.

This entry reviews three types of statistical approaches used in GE interaction for breeding and variety development: (1) regression on the environmental mean, best known as Finlay–Wilkinson regression, or joint regression analysis; (2) linear-bilinear models, like AMMI and GGE; and (3) factorial regression models (see specific references for these methods below). These methods differ not only on the information they provide, but also in their predictive ability for breeding. A discussion of these three types of models from a common statistical perspective can be found in [120, 121]. The approaches aim at substituting the $(GE)_{ij}$ term by a linear or bilinear approximation using fewer parameters (Table 1). The replacement of double-indexed ANOVA GE interaction parameters by single-indexed regression and bilinear parameters introduces predictive properties.

Regression on the Mean

The most widely used and abused statistical method in breeding programs for characterizing GE has been the regression-on-the-mean analysis first proposed by Yates and Cochran [122] and made popular by Finlay and Wilkinson [123] (FW), and also named joint regression analysis. This method summarizes phenotypic responses to environmental changes as straight lines differing in both intercept (related to genotypic main effect) and slope (which estimates environmental sensitivity); GE interaction is revealed by differences in the slopes of individual genotypes. These straight lines are produced upon regressing individual genotypic means per environment on average site performance across all genotypes in that environment, where the regression is done across the full set of environments.

The rationale behind FW is that in the absence of explicit environmental information, a good estimate of the agronomical value of any environment may be given by the average phenotypic performance of all genotypes in that environment. This method has an important conceptual drawback. Two environments may have a similar low average yield for two completely different agroecological reasons, for example, presence of a disease and an episode of a late spring frost just before flowering. This model assumes the genotypic sensitivity to these two stresses to be approximately the same when the different stresses produce the same environmental means. Therefore, the use of the model is best restricted to those rare cases in which environmental differences are driven by just a single major biotic or abiotic factor; in these cases, the linear regression on the mean model may reflect linear differences in relation to the predominant stress factor. However, if environmental differences are due to a major stress, why not using, rather than the average phenotypic value at every environment, a direct estimate of the genotypic sensitivity to this stress as in the factorial regression method described below?

Regression-on-the-mean models are conceptually simple: The differential genotypic responses are summarized by their slopes, but it is very important to point out that their value and use should depend on the proportion of GE sum of squares that can be described by the differential environmental sensitivities of the genotypes. Figure 4 presents an example for which the Finlay and Wilkinson model should have never been used; however, it has been presented in this entry as similar reports are still too often seen in many publications. It summarizes a small MET consisting of seven barley varieties (Var_1 to Var_7) grown at ten Spanish environments according to model III in Table 1. In the part of this figure, there are the simple linear regression models for the seven varieties. If nothing else is shown, it can be wrongly assumed that there are substantial differences among genotypic slopes. This is also shown on the top table that includes

Genotype by Environment Interaction and Adaptation. Table 1 Overview of statistical models for GE analyses from two-way genotype by environment table of means derived from MET

General model	Specific model	Model	Data required	Statistical models for $E(Y_{ij}) - \mu$	Key information provided[a]
Reference models	*Additive*	I	Phenotypic data[b]	$G_i + E_j + e_{ij}$	Average cultivar yields
	Full interaction	II	Phenotypic data	$G_i + E_j + (GE)_{ij}$	Departures from additivity for each environment
Regression on the mean	*Finlay and Wilkinson*	III	Phenotypic data	$G_i + E_j + \beta_i E_j + e_{ij}$	Cultivar sensitivity (in form of slopes) to changes in environmental productivity
Bilinear models	*AMMI*	IV	Phenotypic data	$G_i + E_j + \sum_{k=1}^{K} a_{ki} b_{kj} + e_{ij}$	Joint adaptation patterns of genotypes to environments
	GGE	V	Phenotypic data	$E_j + \sum_{k=1}^{K} a'_{ki} b'_{kj} + e_{ij}$	Identification of the "winning genotype" for each uniform subset of environments
Factorial regression models	*Factorial regression model*	VI	Phenotypic and environmental data	$G_i + E_j + \beta_i z_j + e_{ij}$	Cultivar sensitivities (β_i) to changes in any environmental variable z
	Genotypic factorial regression model: QTL.E model	VII	Phenotypic and genotypic (marker information) data	$x_i\rho + E_j + x_i\rho_j + e_{ij}$	Marker (x) potentially associated to QTL and to QTL.E and the corresponding QTL (ρ) and the QTL.E (ρ_j) effects[c]
	Integrated factorial regression model	VIII	Phenotypic, genotypic, and environmental data	$x_i\rho + E_j + x_i(\lambda z_j) + e_{ij}$	QTL sensitivity to changes in environmental variable z[d]

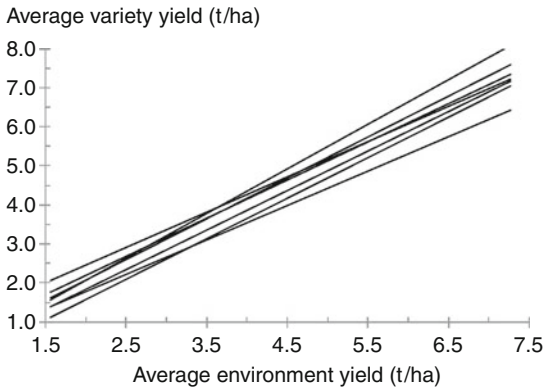
[a]See text for a more detailed discussion of each model

[b]Phenotypic response of the $i = 1...g$ genotype at the $j = 1...e$ environment

[c]In the presence of QTL.E, ρ_j adjusts the average QTL expression across environments, ρ, to a more appropriate level for the individual environment j. This model can be easily extended to x_s markers throughout all the genome

[d]λ is a constant that determines the extent to which a unit change in z, an environmental covariable, influences the effect of a QTL allele substitution. This model can be easily extended to x_s markers and z_t environmental variables

regression estimates. When independent simple linear regression analyses are fitted for the seven genotypes, the slopes varied from 0.88 to 1.14 and the individual straight lines were very significant (R^2 from 84% to 98%; p values from 1.8×10^{-04} to 7.1×10^{-08}). However, these R^2s do not mean anything in the GE context. They simply confirm that the genotypic yield increases with the mean environmental yield, which is obvious in the way that this model is built. Based on these estimates, it can be wrongly stated, for example, that Var_3 (slope equal to 1.14) apparently benefits more to improvements in the overall productivity of the environment than Var_6 (1.01) and particularly than Var_2 (0.88) which, with the lowest sensibility, does worst than expected. However, this model is completely inadequate for this MET and the previous estimates are useless and misleading and should have never been determined. The standard errors of the slopes, which can be used to assess the significance of the differences among slopes, ranges from 0.06 to 0.14, with an average standard error of the difference equal to 0.16. They are too large for detecting significant



Estimates	Var_1	Var_2	Var_3	Var_4	Var_5	Var_6	Var_7
Mean (t/ha)	4.21	3.6	4.41	4.32	3.71	3.92	4.23
Slope (s.e.)	0.98 (0.08)	0.88 (0.11)	1.14 (0.06)	0.90 (0.14)	1.04 (0.14)	1.01 (0.09)	1.05 (0.08)
R^2	0.95	0.88	0.98	0.84	0.88	0.94	0.95

Source of variation	Degrees of freedom	Sum of Squares	Semipartial R^2 (%)	Mean Squares	F-value	*p*-value
E	9	210.58	89.0	23.40	63.08	<0.0001
G	6	5.97	2.5	1.00	2.68	0.0237
GE	54	20.03	8.5	0.37		
Heterogenity of slopes	*6*	*1.42*	*7.1*	*0.24*	*0.61*	*0.7207*
Residual	*48*	*18.61*	*92.9*	*0.39*		

Genotype by Environment Interaction and Adaptation. Figure 4
Inappropriate use of the Finlay and Wilkinson analysis for a MET consisting of seven barley genotypes grown in ten environments in Spain

differences between genotypic slopes. Furthermore, joint regression analysis of variance table (bottom part of Fig. 4) shows that the observed differences among the genotypic slopes (Heterogeneity of slopes) only explains 7.1% of the GE sum of squares, which is not statistically significant (p value = 0.721).

Bilinear Models (AMMI and GGE)

The usefulness of the integration of ecophysiological and statistical tools in the interpretation of GE interaction is examined based upon the joint application of two multiplicative models for interaction: the additive main effects and multiplicative interaction (AMMI) model [6], and the factorial regression model [120, 124]. Both provide information and insight beyond the classical analysis of variance of two-way genotype by environment tables. AMMI represents an empirical approach (based on yield itself) to analyze GE interaction. Factorial regression attempts to describe interaction by including external genetic, phenotypic, and environmental information (e.g., morphophysiological traits, climatic data, etc.) on the levels of the genotypic and environmental factors. It implies a more analytical approach to the understanding of GE.

The Finlay and Wilkinson model belongs to a wider class of statistical models named linear-bilinear which estimate genotypic sensitivities to one or more environmental characterizations that are just linear functions of the phenotypic data [124–127]. However, the additive main effects and multiplicative interaction (AMMI) model [128–131] and the GGE models [132, 133] represent more powerful, and thus, useful examples of linear-bilinear models in plant breeding. These two model classes generate for every genotype and for every environment a series of K scores, which

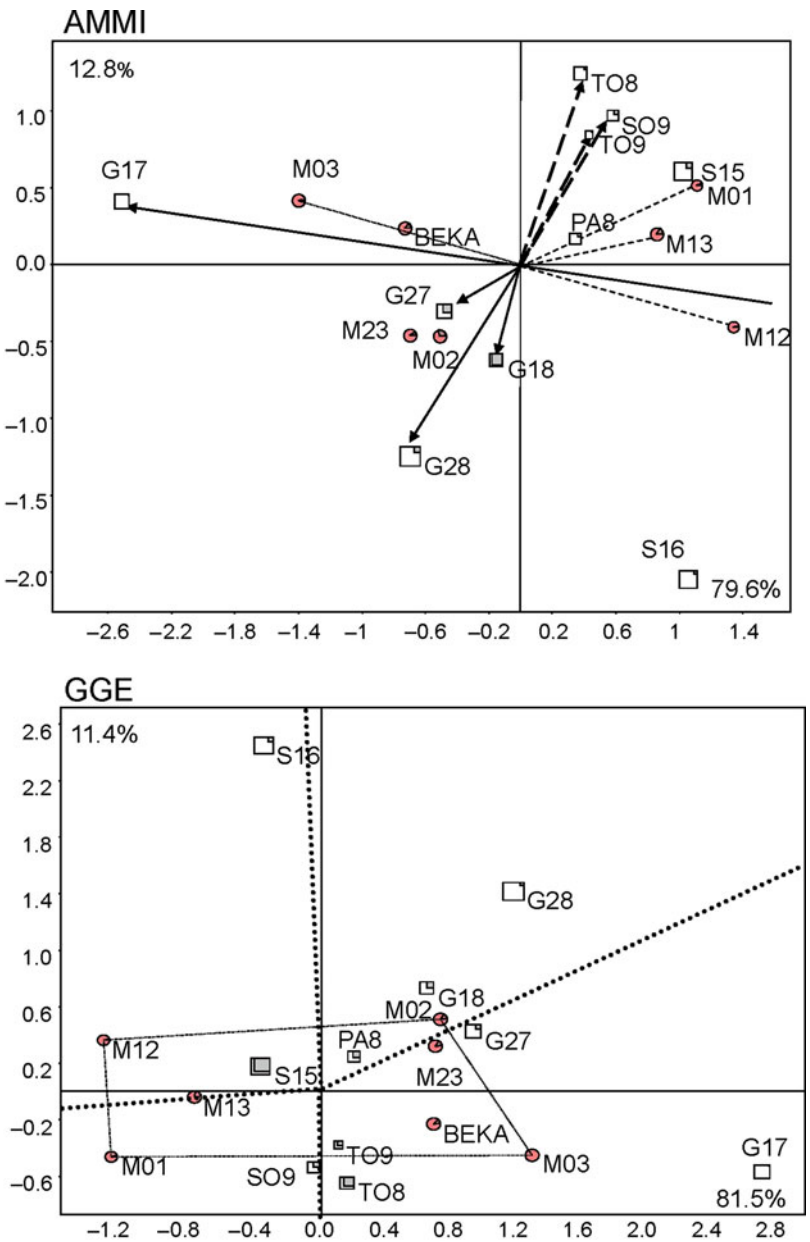
summarize the differential sensitivity of the genotypes to the prevalent, and typically unknown, stresses present in the analyzed MET.

The AMMI model successively partitions the $(GE)_{ij}$ interaction term from the basic ANOVA reference model into a series of K multiplicative terms or products of the form $a_{ki}b_{kj}$, where, for the kth term, a_{ki} refers to the genotypic sensitivity of genotype i to an hypothetical environmental variable b_k, which has value b_{kj} in environment j (Table 1, model IV). Alternatively, b_{kj} refers also to the environmental potentiality of environment j to an hypothetical genotypic variable a_k, which takes value a_{ki} for genotype i. The K hypothetical environmental (genotypic) variables have the property of discriminating maximally between genotypes (environments). The number of multiplicative terms to be retained for an appropriate estimate of the GE interaction, K, can be estimated in various ways, see, for example, Gollob [130], Gauch [6], and Cornelius [134]. From a practical point of view, the AMMI model is fitted in two steps. First, an additive ANOVA model is fitted containing the main effects for G and E and then the residuals from the additive model are used to construct the GE interaction matrix. This interaction matrix is then subjected to a singular value decomposition that generates the above-introduced genotypic and environmental scores [128, 130, 131].

Key outputs of the AMMI analysis are the genotypic and environmental scores for the K retained axes, along with the proportions of the interaction sum of squares explained by the multiplicative terms. The output of the $K = 2$ AMMI model, retaining just the first two interaction axes (IPCA1 and IPCA2), can be directly visualized by means of a biplot [5, 128, 135]. If both axes together explain most of the GE interaction, interpretation of the biplot is very simple and potentially extremely useful for understanding GE interaction. The ith genotype is placed in the biplot according to the (a_{1i}, a_{2i}) genotypic scores; similarly, the jth environment is defined by its two IPCA environmental scores (b_{1j}, b_{2j}). Distance of a genotype or environment to the origin is proportional to the GE interaction generated by that genotype or environment, respectively. Genotypes placed close together show similar adaptation patterns. Close environments generate similar GE interactions.

The actual interaction of genotype i in environment j can be estimated by the projection of the genotype position (a_{1i}, a_{2i}) on the jth environmental vector that goes from the origin (0,0) to (b_{1j}, b_{2j}), that is the line that goes through the origin with slope equal to b_{2j}/b_{1j}. The distance between the genotype projection on the line to the origin also provides information about the absolute magnitude of the interaction of genotype i in environment j. Genotype i will be well adapted to environment j, that is, positive interaction, if the projection is in the direction of the environmental vector and negative otherwise. The sign of the interaction of the genotype i in environment j can be estimated by the cosine between the ith genotypic and the jth environmental vector. It will be positive if both vectors form acute (close to 0°) angles, negative if the angle is obtuse (close to 180°), and nonexistent (no interaction) if they form a right angle (close to 90°). In a similar way, two environments whose vectors form an acute angle generate a similar type of GE interaction across genotypes, the environments have positive genetic correlation. If the two environmental vectors form an angle close to 180°, whichever genotype is well adapted in one environment will be poorly adapted to the other, the environments have a negative environmental interaction. Finally if both environmental vectors form a right angle, the genotypic behavior at one environment will be independent of the behavior at the other site, the genetic correlation is zero.

The upper part of Fig. 5 shows an AMMI biplot generated by a set of seven genotypes grown at ten environments. The genotypes are shown by circles and they represent a barley variety Beka, three derived single nonallelic mutants, M01, M02, M03, and the three binary mutant combinations, M12, M13, M23. The environments are shown in the biplot by squares which represent location by year combinations across Spain. Production of these mutants and analysis of these data was presented elsewhere [136, 137]. In this MET, the GE interaction is well described by the AMMI $K = 2$ model, as both axes explain together more than 90% of the GE sum of squares. The average yield of each environment and genotype is shown proportional to the area of its corresponding symbol. Within each symbol there is a, generally small, darker sector that represents the proportion of its sum of squares not



Genotype by Environment Interaction and Adaptation. Figure 5

AMMI and GGE biplots for a MET consisting of seven barley genotypes grown at ten environments in Spain (Data taken from [105]). See text for a detailed description of genotypes and environments

explained by this model. In this case all environments are well represented except for G27 y G18, which generate GE interactions not correctly described by the AMMI $K = 2$ model. Beka is placed close to the origin and, thus, it is the genotype that interacts least with the ten environments; on the contrary, M12 and M03 are the two genotypes that interact most with the environments. G17 and S16 are the two environments which showed the largest GE interaction, that is, whose genotypic yields depart most from their averages. PA8, near the origin, produced yields close to the average across all environments.

The relative position of both genotypes and environments can provide some clarification on the nature of the GE interactions in this MET. The first IPCA seems to be associated with differential behavior of genotypes carrying the first mutation, M01, M12, and M13, with positive scores in comparison to the other genotypes. These mutants are particularly poorly adapted to Granada (G in the biplot, especially G17). The second axis, which is quantitatively less important, seems associated with mutant 2 (M02, M12 y M23), which shows negative scores on this axis, whereas the other genotypes have positive scores; the specific adaptation of this mutant to the environments is not as clear.

The angle formed by any two environmental vectors is related to the relative similarity among environments, say, the genetic correlation, as determined by the genotypic yields. In this case, the relative yields of the genotypes in Toledo (TO8 y TO9) seem very similar to Soria (SO8). They all form acute angles with cosine and correlation close to 1. T09, with a smaller size square, had lower yields that the others. By comparing the angle of these three environmental vectors with the vector determined by G28 (very obtuse angle closed to 180° and cosine and correlation close to −1), it can be deduced that those genotypes that behave relatively well in G28 perform poorly in the other three sites and vice versa. The analysis of the genotypic projection on environmental vectors gives clues about specific adaptation patterns. For example for G17, M03 showed a good adaptation to this environment, whereas M12 was particularly poorly adapted there. This AMMI analysis was done on the MET data used for the analysis in Fig. 4. Whereas the Finlay and Wilkinson method was able to explain only 7% of the GE sum of squares, the AMMI model for $K = 2$ retained 90% of the GE sum of squares. Furthermore, as described in the previous paragraphs, the known structure of the seven genotypes developed through artificial mutagenesis, suggested a model with a plausible genetic meaning.

The environmental and genetic scores are simple statistical estimates derived from MET phenotypic data, without any direct physiological meaning. However, these empirical estimates can be associated to physiological processes by correlating the environmental scores to explicit environmental measurements, such as soil or meteorological variables; these correlations can often provide meaningful agroecological information about the nature of GE interactions [11, 14, 138–140].

Another member of the linear-bilinear model class is the GGE model [132, 133], in which single value decomposition is done on the sum of the G and GE components by just subtracting the environmental means (environmental centered) on the two-way table of means (Table 1, model V) rather than on GE interactions alone, as done in AMMI. A GGE biplot for $K = 2$ provides additional information of potential interest to breeders, as it allows for the direct identification of the "winning" genotype in any potentially uniform subset of environments. To do so, the most extreme genotypic scores are connected delimiting an irregular polygon enclosing all other genotypes, that is, a convex hull is constructed. In the previous example (Fig. 5, bottom) this is an irregular quadrilateral defined by M12, M02, M03 y M01. Next, lines perpendicular to each side of the polygon/convex hull are drawn (thicker lines in Fig. 5, bottom) up to the boundaries of the biplot. In this way sectors are created, called mega environments, which contain environments that behave relatively uniform with respect to the genotypes. The "winning genotype" in a mega environment is the genotype that is placed at the vertex of the polygon inside that mega environment. For example, M12 is the best-adapted genotype in the mega environment defined by S15 and, particularly, S16. Mutant M03 is the most productive genotype in G18 and G28. Of course, this interpretation is subjected to the condition that most of G+GE variability is retained in the first two GGE axes.

Factorial Regression Models

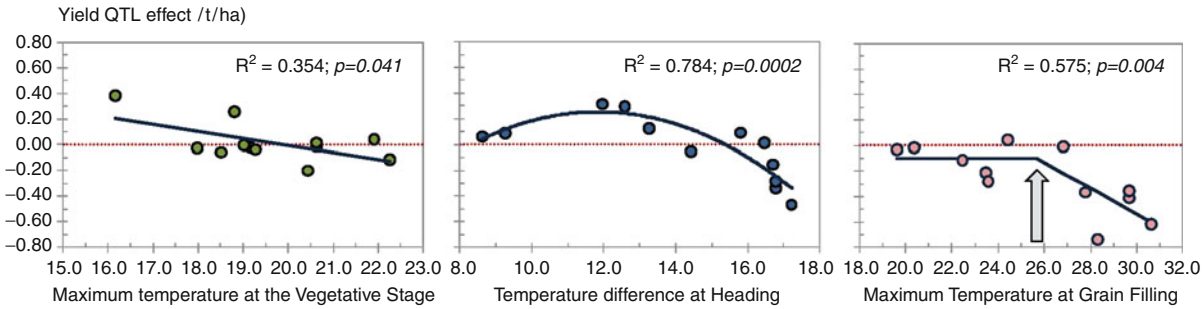
Factorial regression models were developed to incorporate additional explicit environmental information (variable z in Table 1 model VI) into a model [120, 121] for GE interaction and estimate the genotypic sensitivity of each of g genotypes (β_i in Table 1 model VI) to these independent variables (regressors, covariables). The regression on the mean or FW analyses reported before may be seen as a specific case of factorial regression, in which the average yield in each environment is used as an explicit environmental characterization. In the general form, any explicit agroecological variable

individually recorded for each environment could be used as independent explanatory variable. Average yield can be a reflection of a certain meteorological variable, such as available soil water. In this situation, this variable recorded for each environment could be used as explanatory independent variable to describe GE interaction (variable z in model VI Table 1). The genotypic slopes will have a more direct physiological meaning when they estimate, for example, sensitivity to changes in available soil water, which is an approximation to water use efficiency. In a triticale MET, GE interaction for grain yield was regressed on soil pH and the genotypic slopes directly assessed the sensitivities to changes in soil pH [141]. Extension to multiple environmental variables and complex response curves is conceptually simple and easily computable using standard statistical packages. As for any multiple regression models, a central question is the choice of variables for description of GE interaction. Continuous monitoring of the environment generates huge numbers of environmental covariables, which will complicate identification of the most relevant ones. Purely statistical selection procedures often lead to physiologically incomprehensible models. Therefore, agroecological insights of genotypes and environments should augment and prevail over purely statistical considerations. A helpful prescreening of environmental covariables can sometimes be done by correlating covariables to scores derived from AMMI or GGE analyses [11].

Factorial Regression Models Incorporating Explicit Genotypic Information Genotypic covariables can also be used to partition the G and GE terms. Molecular markers such as DNA polymorphisms for anonymous sequences or for functional genes are the most useful and readily available genetic covariables. For a codominant marker in a diploid species with potential genotypes *AA*, *Aa*, and *aa*, the number of *A* alleles (2, 1, and 0 to represent genotypes *AA*, *Aa*, and *aa*, respectively) could be used as a genetic covariable, x, in a factorial regression model (Table 1, model VII). If multiple markers across the whole genome are sequentially used, factorial regression has the ability to detect, locate, and estimate QTL main effects and QTL by environment interactions. For marker positions adjacent to a QTL, the ρ slope in model VII (Table 1) estimates directly the effect of a QTL allele substitution. Similarly, the $(GE)_{ij}$ interaction can be further partitioned into a term for differential QTL expression across environments, ρ_j, and a residual GE interaction. For a full genome scan, factorial regression models can be fitted on grid of genomic positions, on markers and in between markers, when necessary. Virtual markers, in between observed markers, can be easily generated from flanking marker information (see [142]). Factorial regression models which include genetic covariables can be potentially used for any set of genotypes for which genetic predictors can be constructed, from standard biparental offspring populations and unrelated diverse association panels, to more complicated intercross systems, such as MAGIC [37], NAM [38, 39], and AMPRIL [40] described before. The QTL.E interaction model shown in model VII (Table 1) is based on application of a simple marker regression to our data. To construct multiple QTL models, a composite interval mapping approach can be followed by incorporating cofactors, or markers that correct for QTL elsewhere, on the genome.

Factorial Regression Models Incorporating Explicit Environmental and Genotypic Information The final goal of any MET is to understand the nature of GE interaction in terms of differential sensitivity of the different QTLs or genes to external environmental variables. This is also possible by means of factorial regression models [11, 13, 72, 92]. Differential QTL expression for environments, ρ_j, can be regressed on any environmental covariable, z, to relate the differential QTL expression directly to key environmental variables responsible for GE. This is done by substituting the QTL.E term, $x_i\rho_j$, with a linear regression $x_i(\lambda z_j)$ and a residual term. λ is a constant that determines the extent to which a unit change in z, the environmental covariable, influences the effect of a QTL allele substitution. The statistical model used is listed as model VIII in Table 1 which can be easily extended to multiple markers (x_{si}) and various environmental variables (z_{tj}).

Van Eeuwijk et al. [143, 144] and Boer et al. [99] provide examples of differential QTL expression in maize data to environmental variables; by incorporating marker information and environmental covariables describing the environment,



Genotype by Environment Interaction and Adaptation. Figure 6

Differential sensitivities of three major QTLs to temperature, recorded at three different growing periods for the Steptoe × Morex doubled haploid population (Data taken from the North American Barley Genome Project). Twelve sites with environmental characterizations were available. Three different models were used: a straight-line regression model, a second-degree polynomial, and a "broken-stick" factorial regression model

these models allow for prediction of differential genotypic sensitivities to environmental changes. An example of the output of these fully integrated genotypic and environmental models is shown in Fig. 6, which shows an analysis for the "Steptoe × Morex" double haploid population data from the North American Barley Genome Project, grown at 12 sites and with environmental covariables at hand. Three main QTLs were responsible of GE interaction [71]. Differential QTL effects across environments could be associated to three different environmental variables related to temperature taken at three different growth periods and according to three alternative models: a simple linear regression model, a second degree response, and a "broken-stick" model (Fig. 6). Furthermore, two out of three QTL.E interactions showed a "crossover" type interaction: The sign of the QTL effect changed according to the value of the environmental external variable. This figure clearly illustrates the importance of QTL.E interaction for complex traits such as grain yield in barley.

The Mixed Model Framework: Modeling Variance-Covariance Structures

Table 1 shows different alternatives for modeling the expected responses of a genotype to environmental changes, without any specific concern about the implicit assumptions of the analyses of variance. Standard linear models take for granted that error terms are independent and have constant variance. However for MET, these assumptions are overly simplistic as variances within environments and correlations between environments tend to be heterogeneous. For the sake of brevity and simplicity, how the mixed model framework also allows for modeling of the variance-covariance component of the data has not been described. However, the optimal statistical modeling for MET data should focus first in finding an adequate variance-covariance model for the random terms and then, as discussed above, search for a parsimonious model for the expected responses. Choice of variance-covariance model can have strong implications. In the case of QTL modeling, QTL may erroneously be declared significant or nonsignificant because of over or under estimation of effect sizes and standard errors [72, 145]. The mixed model framework, which combines modeling of means and variances, provides a more appropriate modeling environment for GE and QTL.E interactions offering flexibility with regard to assumptions on heterogeneity of variances and on correlations across environments [17].

Computer Software for GE Analyses

Annicchiarico [3] lists a series of user-friendly computer software available for many GE analyses. CROPSTAT is a freely available package developed by the International Rice Research Institute [146] that has specific modules for FW and AMMI analysis. MATMODEL available in a free version [147] also provides AMMI and joint regression modeling and it

is particularly useful for handling missing data. INFOGEN [148] within the INFOSTAT system [149] also includes most described tools for the analysis of MET. At the same time, there are also dedicated commercial softwares, such as GGE BIPLOT [132], useful for joint regression, AMMI, and GGE. Obviously, all general statistical packages can easily be programmed to fit all linear-bilinear models described in this entry in a fixed model context, whereas some like GenStat, ASREML, and SAS also allow fitting mixed bilinear models. SAS instructions for many GE analyses are presented in Kang [100]. GenStat [150] includes specific procedures for FW, AMMI, and GGE analyses. Version 13 of GenStat (2010) also includes dedicated menus for QTL and QTL.E analyses for segregating crosses and for association analyses. GenStat has a policy of free licensing of older versions to institutions in developing countries and for educational purposes in the form of the GenStat Discovery version.

Future Directions

Plant breeding research experiences fast changes. Nowadays, at the genomic side, sequencing and single-nucleotide polymorphism (SNP) technology is becoming increasingly cheap for not only model species, but also for crop species. Besides information at the DNA level, genomic information at RNA, protein, and metabolite level starts to become common. As a consequence, huge amounts of data start to become available for characterizing genotypes at various genomic levels. Similar developments can be observed for monitoring the environment. Environmental characterizations can be stored over the growing season for all environmental factors that are believed to be relevant.

In the past, genotypic and environmental information was the bottleneck; however, the current focus has shifted to access to the right plant material and their correct phenotyping. High-throughput phenotyping techniques are being developed that facilitate monitoring of individual plants at arbitrary small intervals over the growing season. However, high-throughput phenotyping schemes taken in individual cell/tissue/organ/single plant level may not mean anything at the crop level. Up-scaling from processes taking place in a fraction of a second and in a fraction of space to relevant crop traits (produced in a hectare through several months) has consistently failed in the past and remains a challenge. Crop physiology can play a key role in understanding multi-trait interactions for up-scaling from gene to crop.

The strongly increased availability of phenotypic, genomic, and environmental information begs for new statistical techniques that allow the increased information to be used in an effective way. Various requirements can be defined. First, phenotypic information will increasingly concern a wide array of traits that are repeatedly measured over time. Correlations between these traits will need to be explicitly modeled, as will be the correlations between the repeated measurements for the same trait. Information from multiple environments can be treated in the same way as information from multiple traits, although correlations between the same trait in different environments may ask for other models than the correlations between different traits in the same environment and different traits in different environments. Standard mixed model procedures will fail, as too many variances and covariances/correlations will require estimation. A way out may be too regularize the pattern of variances and covariances by inserting biological information in the estimation in the form of alternative statistical tools, such as priors (Bayesian methods) or penalties (penalized multivariate regressions). One popular way of reducing the number of correlation parameters is by imposing network structures on sets of trait by environment combinations, thereby effectively fitting sparse matrices to the inverses of the correlation matrices. The graphical lasso is an example of such an approach [151].

Turning to increased marker numbers and selecting meaningful genotype to phenotype models in the face of 100,000s of SNP markers demand new statistical approaches. As identification of individually contributing SNPs in such conditions is very difficult, an alternative strategy emphasizing prediction from markers above identification of markers is rapidly gaining popularity. In genomic selection, the idea is to use all markers simultaneously for predicting marker-based breeding values that help in ranking individuals on genetic merit [152–154]. Bayesian and penalized regression techniques help to regularize the estimates for individual marker contributions, as it will be evident that with standard regression techniques it is impossible to estimate hundreds of thousands of

marker effects. Mixed models can in this context be interpreted as an example of a Bayesian technique in which the prior for the marker effects is a normal distribution. Equivalently, mixed models can be seen as penalized regressions in which the ratios of variance components determine the penalties (shrinkage factors). As an illustration, one may regress a phenotypic trait on a large set of markers, assuming the effects of the markers of individual chromosomes to follow normal distributions with chromosome-specific variances. The predicted values for the genotypes from such a mixed model represent the genomic breeding value. This breeding value can be used for selection purposes. Examples of genomic selection for multiple environments are still hard to find.

The increased information from intensive environmental monitoring can be used to improve prediction of genotypic performance by integrating it with other types of genotype-specific information in crop growth models [16, 17, 155–157]. The environmental information is fed into a suitable crop growth model and when physiological parameters of the crop growth model can be specified at genotype-specific level, the crop growth model can produce predictions for individual genotypes in any environment for which a full environmental characterization is given. An integration of crop growth modeling with genomic selection is possible when the values for the genotype-specific physiological parameters in the crop growth model are inserted from Bayesian or mixed genomic selection models.

The increased amounts of phenotypic, genomic, and environmental data pose strong demands on our statistical ingenuity, but interesting solutions start to appear on the horizon. In this forthcoming scenario, elaborations of mixed models, Bayesian techniques and penalized methods will play a major role in the analysis of GE interactions.

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Global Economic Impact of Transgenic/Biotech Crops (1996–2008)

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Article Outline

Glossary

Direct farm income benefit Improvements in income arising from changes in yield and production levels or associated with cost reductions/productivity enhancements associated with the use of transgenic crops.

Herbicide tolerance Tolerance to a herbicide (e.g., glyphosate) delivered by genetic modification techniques. This allows a crop to be sprayed with the "tolerant herbicide" without harming the crop but providing good weed control.

Insect resistance Resistance to a pest (e.g., corn-boring pests) delivered by genetic modification techniques. This allows a crop to be grown without having to use alternative methods of pest control, notably the use of insecticides.

Nonpecuniary benefit Additional farm-level benefits to direct farm income benefits that are more intangible and difficult to measure in monetary terms (e.g., additional management flexibility).

No tillage agriculture The use of a production technique in which the soil is not tilled/plowed. It is in contrast to traditional plow-based production systems and allows farmers to save on fuel use and contributes to improved soil water retention and reduced soil erosion.

Second crop soybeans The planting of a crop of soybeans after another crop (often wheat) in the same growing season. This allows a farmer to obtain two crops from the same piece of land in one season.

Definition of the Subject

The application of biotechnology to commercial agriculture on a widespread basis has occurred since 1996. The extent of this adoption in terms of crops and (biotechnology) traits is explored and the associated economic impacts for the period 1996–2008 are assessed, to help identify some of the main reasons why farmers have adopted the technology.

Introduction

This article examines specific global socioeconomic impacts on farm income over the 13-year period 1996–2008. It also quantifies the production impact of the technology on the key crops in areas where it has been used. The analysis concentrates on farm income effects because this is a primary driver of adoption among farmers (both large commercial and small-scale subsistence). It also considers more indirect farm income or nonpecuniary benefits, and quantifies the (net) production impact of the technology. More specifically, it covers the following main issues:

- Impact on crop yields
- Effect on key costs of production, notably seed cost and crop protection expenditure
- Impact on other costs such as fuel and labor
- Effect on profitability
- Other impacts such as crop quality, scope for planting a second crop in a season and impacts that are often referred to as intangible impacts such as convenience, risk management, and husbandry flexibility
- Production effects

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The contribution is based largely on extensive analysis of existing farm-level impact data for biotech crops. While primary data for impacts of commercial cultivation were not available for every crop, in every year and for each country, a substantial body of representative research and analysis is available and this has been used as the basis for the analysis presented.

As the economic performance and impact of this technology at the farm level varies widely, both between, and within regions/countries (as applies to any technology used in agriculture), the measurement of performance and impact is considered on a case-by-case basis in terms of crop and trait combinations. The analysis presented is based on the average performance and impact recorded in different crops by the studies reviewed; the average performance being the most common way in which the identified literature has reported impact. Where several pieces of relevant research (e.g., on the impact of using a GM trait on the yield of a crop in one country in a particular year) have been identified, the findings used have been largely based on the average of these findings.

This approach may both, overstate, or understate, the real impact of GM technology for some trait, crop and country combinations, especially in cases where the technology has provided yield enhancements. However, as impact data for every trait, crop, location, and year is not available, the authors have had to extrapolate available impact data from identified studies to years for which no data are available. Therefore, the authors acknowledge that this represents a weakness of the research. To reduce the possibilities of over/understating impact, the analysis:

- Directly applies impacts identified from the literature to the years that have been studied. As a result, the impacts used vary in many cases according to the findings of literature covering different years. Hence, the analysis takes into account the variation in the impact of the technology on the yield based on its effectiveness in dealing with (annual) fluctuations in pest and weed infestation levels as identified by research.
- Uses current farm-level crop prices and bases any yield impacts on (adjusted – see below) current average yields. In this way, some degree of dynamic has been introduced into the analysis that would, otherwise, be missing if constant prices and average yields indentified in year-specific studies had been used.
- Includes some changes and updates to the impact assumptions identified in the literature based on consultation with local sources (analysts, industry representatives) so as to better reflect prevailing/changing conditions (e.g., pest and weed pressure, cost of technology).
- Adjusts downward the average base yield (in cases where GM technology has been identified as having delivered yield improvements) on which the yield enhancement has been applied. In this way, the impact on total production is not overstated.

Other aspects of the methodology used to estimate the impact on direct farm income are as follows:

- Impact is quantified at the trait and crop level, including where stacked traits are available to farmers. Where stacked traits have been used, the individual trait components were analyzed separately to ensure estimates of all traits were calculated.
- All values presented are nominal for the year shown and the base currency used is the US dollar. All financial impacts in other currencies have been converted to US dollars at prevailing annual average exchange rates for each year.
- The analysis focuses on the changes in farm income for each year, arising from the impact of GM technology on yields, key costs of production, notably seed cost and crop protection expenditure and also the impact on costs such as fuel and labor (inclusion of impact on these categories of cost are, however, more limited than the impacts on seed and crop protection costs because only a few of the papers reviewed have included consideration of such costs in their analyses). Therefore, in most cases the analysis relates to impact of crop protection and seed cost only.
- Crop quality (e.g., improvements in quality arising from less pest damage or lower levels of weed impurities that result in price premia being obtained from buyers) and the scope for facilitating the planting of a second crop in a season (e.g., second crop soybeans in Argentina following wheat that would, in the absence of the GM herbicide-tolerant

(GM HT) seed, probably not have been planted). Thus, the farm income effect measured is essentially a gross margin impact (impact on gross revenue less variable costs of production) rather than a full net cost of production assessment. Through the inclusion of yield impacts and the application of actual (average) farm prices for each year, the analysis also indirectly takes into account the possible impact of biotech crop adoption on global crop supply and world prices.

This article also examines some of the more intangible (more difficult to quantify) economic impacts of GM technology. The literature in this area is much more limited and in terms of aiming to quantify these impacts, largely restricted to the US-specific studies. The findings of this research (notably relating to the USA, and drawing on Marra and Piggot [1, 2] are summarized and extrapolated to the cumulative biotech crop planted areas in the USA over the period 1996–2008.

Lastly, this article includes estimates of the production impacts of GM technology at the crop level. These have been aggregated to provide the reader with a global perspective of the broader production impact of the technology. These impacts derive from the yield impacts (where identified), but also from the facilitation of additional cropping within a season (notably in relation to soybeans in South America).

Economic Impact of Transgenic/Biotech Crops

The section below is structured on a trait and country basis highlighting the key farm-level impacts.

Herbicide-Tolerant Soybeans

The USA

In 2008, 92% of the total US soybean crop was planted to genetically modified herbicide-tolerant cultivars (GM HT). The farm-level impact of using this technology since 1996 is summarized in Table 1.

The key features are as follows:

- The primary impact has been to reduce the soybean cost of production. In the early years of adoption, these savings were between $25/ha and $34/ha. In recent years, estimates of the cost savings have been in the range of $30–$85/ha (based on a comparison of conventional herbicide regimes in the early 2000s that would be required to deliver a comparable level of weed control to the GM HT soybean system). In 2008, the cost savings declined relative to earlier years because of the significant increase in the global price of glyphosate relative to increases in the price of other herbicides (commonly used on conventional soybeans). The main savings have come from lower herbicide costs (while there were initial cost savings in herbicide expenditure, these increased when glyphosate came off-patent in 2000. Growers of GM HT soybeans initially applied Monsanto's Roundup herbicide but over time, and with the availability of low-cost generic glyphosate alternatives, many growers switched to using these generic alternatives (the price of Roundup also fell significantly post 2000) plus a $6–$10/ha savings in labor and machinery costs.
- Against the background of underlying improvements in average yield levels over the 1996–2008 period (via improvements in plant breeding), the specific yield impact of the GM HT technology used up to 2008 has been neutral (some early studies of the impact of GM HT soybeans in the USA, suggested that GM HT soybeans produced lower yields than conventional soybean varieties. Where this may have occurred, it applied only in early years of adoption when the technology was not present in all leading varieties suitable for all of the main growing regions of the USA. By 1998/1999, the technology was available in leading varieties and no statistically significant average yield differences have been found between GM and conventional soybean varieties.
- The annual total national farm income benefit from using the technology rose from $5 million in 1996 to $1.42 billion in 2007. In 2008, the farm income was about $1.2 billion. The cumulative farm income benefit over the 1996–2008 period (in nominal terms) was $11 billion.
- In added value terms, the increase in farm income in recent years has been equivalent to an annual increase in production of between +5% and +10%.

Argentina

As in the USA, GM HT soybeans were first planted commercially in 1996. Since then, use of the technology

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 1 Farm-level income impact of using GM herbicide-tolerant (GM HT) soybeans in the USA 1996–2008

Year	Cost savings ($/ha)	Net cost saving/increase in gross margins, inclusive of cost of technology ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1996	25.2	10.39	5.0	0.03
1997	25.2	10.39	33.2	0.19
1998	33.9	19.03	224.1	1.62
1999	33.9	19.03	311.9	2.5
2000	33.9	19.03	346.6	2.69
2001	73.4	58.56	1,298.5	10.11
2002	73.4	58.56	1,421.7	9.53
2003	78.5	61.19	1,574.9	9.57
2004	60.1	40.33	1,096.8	4.57
2005	69.4	44.71	1,201.4	6.87
2006	57.0	32.25	877.1	4.25
2007	85.2	60.48	1,417.2	6.01
2008	68.6	43.88	1,219.5	4.25

Sources and notes:

1. Impact data 1996–1997 based on Marra et al [3], 1998–2000 based on Carpenter and Gianessi [4] and 2001 [5] onward based on Sankala and Blumenthal [6, 7] and Johnson and Strom [8] plus updated 2008 to reflect recent changes in herbicide prices
2. Cost of technology: $14.82/ha 1996–2002, $17.3/ha 2003, $19.77/ha 2004, $24.71/ha 2005 onward
3. The higher values for the cost savings in 2001 onward reflect the methodology used by Sankala and Blumenthal, which was to examine the conventional herbicide regime that would be required to deliver the same level of weed control in a low/reduced till system to that delivered from the GM HT no/reduced till soybean system. This is a more robust methodology than some of the more simplistic alternatives used elsewhere. In earlier years, the cost savings were based on comparisons between GM HT soy growers and/or conventional herbicide regimes that were commonplace prior to commercialization in the mid-1990s when conventional tillage systems were more important

has increased rapidly and almost all soybeans grown in Argentina are GM HT (99%). Not surprisingly, the impact on farm income has been substantial, with farmers deriving important cost saving and farm income benefits both similar and additional to those obtained in the USA (Table 2). More specifically, it covers the following main issues:

- The impact on yield has been neutral (i.e., no positive or negative yield impact).
- The cost of the technology to Argentine farmers has been substantially lower than in the USA (about $1–$4/ha compared to $15–$25/ha in the USA: see Table 1) mainly because the main technology provider (Monsanto) was not able to obtain patent protection for the technology in Argentina. As such, Argentine farmers have been free to save and use biotech seed without paying any technology fees or royalties (on farm-saved seed) for many years and estimates of the proportion of total soybean seed used that derives from a combination of declared saved seed and uncertified seed in 2008 were about 75% (i.e., 25% of the crop was planted to certified seed).
- The savings from reduced expenditure on herbicides, fewer spray runs, and machinery use have been in the range of $24–$30/ha, although in

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 2 Farm-level income impact of using GM HT soybeans in Argentina 1996–2008

Year	Cost savings ($/ha)	Net saving on costs (inclusive of cost of technology ($/ha)	Increase in farm income at a national level ($ millions)	Increase in farm income from facilitating additional second cropping ($ millions)
1996	26.10	22.49	0.9	0
1997	25.32	21.71	42	25
1998	24.71	21.10	115	43
1999	24.41	20.80	152	118
2000	24.31	20.70	205	143
2001	24.31	20.70	250	273
2002	29.00	27.82	372	373
2003	29.00	27.75	400	416
2004	30.00	28.77	436	678
2005	30.20	28.96	471	527
2006	28.72	26.22	465	699
2007	28.61	26.11	429	1,134
2008	16.37	13.87	233	765

Sources and notes:

1. The primary source of information for impact on the costs of production is Qaim and Traxler [9, 10]. This has been updated in recent years to reflect changes in herbicide prices
2. All values for prices and costs denominated in Argentine pesos have been converted to US dollars at the annual average exchange rate in each year
3. The second cropping benefits are based on the gross margin derived from second crop soybeans multiplied by the total area of second crop soybeans (less an assumed area of second crop soybeans that equals the second crop area in 1996 – this was discontinued from 2004 because of the importance farmers attach to the GM HT system in facilitating them remaining in no tillage production systems). The source of gross margin data comes from Grupo CEO
4. Additional information is available in Appendix 1
5. The net savings to costs understate the total gains in recent years because two thirds to 80% of GM HT plantings have been to farm-saved seed on which no seed premium was payable (relative to the $3-$4/ha premium charged for new seed)

2008, savings fell back to about $16/ha because of the significant increase in the price of glyphosate relative to other herbicides. Net income gains have been in the range of $21–$29/ha, although in 2008 a lower average level of about $14/ha has occurred.

- The price received by farmers for GM HT soybeans in the early years of adoption was, on average, marginally higher than for conventionally produced soybeans because of lower levels of weed material and impurities in the crop. This quality premia was equivalent to about 0.5% of the baseline price for soybeans.
- The net income gain from the use of the GM HT technology at a national level was $233 million in 2008. Since 1996, the cumulative benefit (in nominal terms) has been $3.57 billion.
- An additional farm income benefit that many Argentine soybean growers have derived comes from the additional scope for second cropping of soybeans. This has arisen because of the simplicity, ease, and weed management flexibility provided by the (GM) technology, which has been an important factor facilitating the use of no and reduced tillage production systems. In turn, the adoption of low/no tillage

production systems has reduced the time required for harvesting and drilling subsequent crops and hence has enabled many Argentine farmers to cultivate two crops (wheat followed by soybeans) in one season. As such, 20% of the total Argentine soybean crop was second crop in 2008 (3.4 million hectares), compared to 8% in 1996. Based on the additional gross margin income derived from second crop soybeans (see Appendix 1), this has contributed a further boost to national soybean farm income of $765 billion in 2008 and $5.19 billion cumulatively since 1996.

- The total farm income benefit inclusive of the second cropping was $998 million in 2008 and $8.76 billion cumulatively between 1996 and 2008.
- In added value terms, the increase in farm income from the direct use of the GM HT technology (i.e., excluding the second crop benefits) in the last 3 years has been equivalent to an annual increase in production of between +2% and +7%. The additional production from second soybean cropping facilitated by the technology in 2008 was equal to 20% of total output.

Brazil

GM HT soybeans were probably first planted in Brazil in 1997. Since then, the area planted has increased to 62% of the total crop in 2008 (until 2003 all plantings were technically illegal).

The impact of using GM HT soybeans has been similar to that identified in the USA and Argentina. The net savings on herbicide costs have been larger in Brazil due to higher average costs of weed control. Hence, the average cost saving arising from a combination of reduced herbicide use, fewer spray runs, labor and machinery savings were between $30/ha and $81/ha in the period 2003–2008 (Table 3). The net cost saving after deduction of the technology fee (assumed to be about $20/ha in 2008) has been between $9/ha and $61/ha in recent years. At a national level, the adoption of GM HT soybeans increased farm income levels by $592 million in 2008. Cumulatively over the period 1997–2008, farm incomes have risen by $2.74 billion (in nominal terms).

In added value terms, the increase in farm income from the use of the GM HT technology in 2008 was equivalent to an annual increase in production of +2.6% (about 1.54 million tons).

Paraguay and Uruguay

GM HT soybeans have been grown since 1999 and 2000 respectively in Paraguay and Uruguay. In 2008, they accounted for 90% of total soybean plantings in Paraguay and 99% of the soybean plantings in Uruguay (as in Argentina, the majority of plantings are to farm saved or uncertified seed). Using the farm-level impact data obtained from the Argentine research [9, 10] – we are not aware of any published country-specific impact research having been conducted in these two countries) and applying this to production in these two countries, Fig. 1 summarizes the national farm-level income benefits that have been derived from using the technology. In 2008, the respective national farm income gains were $58.8 million in Paraguay and $7.9 million in Uruguay.

Canada

GM HT soybeans were first planted in Canada in 1997. In 2008, the share of total plantings accounted for by GM HT soybeans was 73% (0.88 million hectares).

At the farm level, the main impacts of use have been similar to the impacts in the USA. The average farm income benefit has been within a range of $14–$40/ha and the increase in farm income at the national level was $12.6 million in 2008 (Table 4). The cumulative increase in farm income since 1997 has been $116 million (in nominal terms). In added value terms, the increase in farm income from the use of the GM HT technology in 2008 was equivalent to an annual increase in production of about 1% (34,500 tons).

South Africa

In 2001, GM HT soybeans were planted commercially in South Africa. In 2008, 184,000 ha (80%) of total soybean plantings were to varieties containing the GM HT trait. In terms of impact at the farm level, net cost savings of between $5/ha and $9/ha have been achieved through reduced expenditure on herbicides (Table 5), although in 2008, with the significant increase in glyphosate prices relative to other herbicides, this has fallen back to $2/ha. At the national level, the increase in farm income was $0.32 million in 2008. Cumulatively, the farm income gain since 2001 has been $4.13 million.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 3 Farm-level income impact of using GM HT soybeans in Brazil 1997–2008

Year	Cost savings ($/ha)	Net cost saving after inclusion of technology cost ($/ha)	Impact on farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1997	38.8	35.19	3.8	0.06
1998	42.12	38.51	20.5	0.31
1999	38.76	35.15	43.5	0.96
2000	65.32	31.71	43.7	0.85
2001	46.32	42.71	58.7	1.02
2002	40.00	36.39	66.7	1.07
2003	77.00	68.00	214.7	1.62
2004	76.66	61.66	320.9	2.95
2005	73.39	57.23	534.6	5.45
2006	81.09	61.32	730.6	6.32
2007	29.85	8.74	116.3	0.68
2008	64.07	44.44	591.9	2.63

Sources and notes:

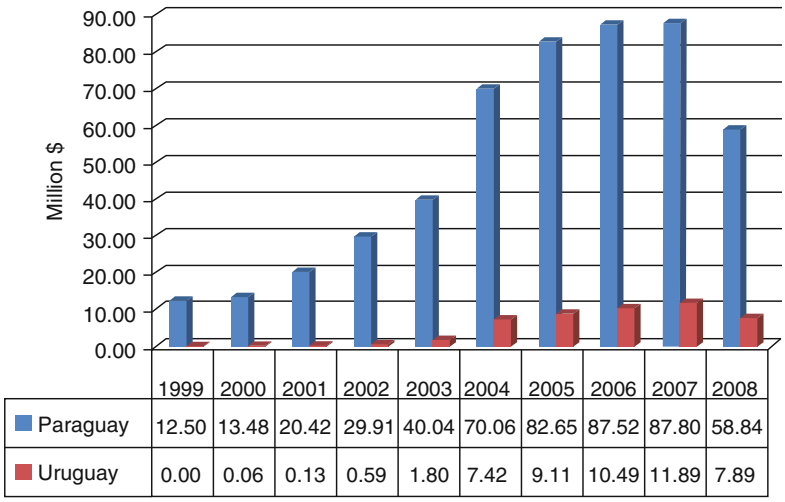
1. Impact data based on 2004 comparison data from the Parana Department of Agriculture [11] Cost of production comparison: biotech and conventional soybeans, in USDA GAIN report BR4629 of 11 November 2004. www.fas.usad.gov/gainfiles/200411/146118108.pdf for the period to 2006 [11]. From 2007 based on Galveo [12]
2. Cost of the technology from 2003 is based on the royalty payments officially levied by the technology providers. For years up to 2002, the cost of technology is based on costs of buying new seed in Argentina (the source of the seed). This probably overstates the real cost of the technology and understates the cost savings
3. All values for prices and costs denominated in Brazilian Real have been converted to US dollars at the annual average exchange rate in each year

Romania

In 2008, Romania was not officially permitted to plant GM HT soybeans, having joined the EU at the start of 2007 (the EU has not permitted the growing of GM HT soybeans to date). The impact data presented below therefore covers the period 1999–2006.

The growing of GM HT soybeans in Romania had resulted in substantially greater net farm income gains per hectare than any of the other countries using the technology:

- Yield gains of an average of 31% have been recorded [14]. This yield gain has arisen from the substantial improvements in weed control (weed infestation levels, particularly of difficult to control weeds such as Johnson grass have been very high in Romania. This is largely a legacy of the economic transition during the 1990s, which resulted in very low levels of farm income, abandonment of land, and very low levels of weed control. As a result, the weed bank developed substantially and has been subsequently very difficult to control, until the GM HT soybean system became available [glyphosate has been the key to controlling difficult weeds like Johnson grass]). In recent years, as fields have been cleaned up of problem weeds, the average yield gains have decreased and were reported at +13% in 2006 (source: farmer survey conducted in 2006 on behalf of Monsanto Romania).
- The cost of the technology to farmers in Romania tended to be higher than other countries, with seed being sold in conjunction with the herbicide.



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 1
National farm income benefit from using GM herbicide-tolerant (GM HT) soybeans in Paraguay and Uruguay 1999–2008 (million $)

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 4 Farm-level income impact of using GM HT soybeans in Canada 1997–2008

Year	Cost savings ($/ha)	Net cost saving/increase in gross margin (inclusive of technology cost: $/ha)	Impact on farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1997	64.28	41.17	0.041	0.01
1998	56.62	35.05	1.72	0.3
1999	53.17	31.64	6.35	1.29
2000	53.20	31.65	6.71	1.4
2001	49.83	29.17	9.35	3.4
2002	47.78	27.39	11.92	2.79
2003	49.46	14.64	7.65	1.47
2004	51.61	17.48	11.58	1.48
2005	55.65	18.85	13.30	2.26
2006	59.48	23.53	17.99	2.22
2007	61.99	24.52	16.87	1.57
2008	56.59	14.33	12.61	1.03

Sources and notes:
1. Impact data based on George Morris Centre Report [13] and updated in recent years to reflect changes in herbicide prices
2. All values for prices and costs denominated in Canadian dollars have been converted to US dollars at the annual average exchange rate in each year

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 5 Farm-level income impact of using GM HT soybeans in South Africa 2001–2008

Year	Cost savings ($/ha)	Net cost saving/increase in gross margin after inclusion of technology cost ($/ha)	Impact on farm income at a national level ($ millions)
2001	26.72	7.02	0.042
2002	21.82	5.72	0.097
2003	30.40	7.90	0.24
2004	34.94	9.14	0.46
2005	36.17	9.12	1.42
2006	33.96	5.17	0.83
2007	32.95	5.01	0.72
2008	25.38	1.77	0.32

Sources and notes:
1. Impact data (Data source: Monsanto South Africa – data provision not a reference)
2. All values for prices and costs denominated in South African Rand have been converted to US dollars at the annual average exchange rate in each year

For example, in the 2002–2006 period, the average cost of seed and herbicide per hectare was $120–$130/ha. This relatively high cost however, did not deter adoption of the technology because of the major yield gains, improvements in the quality of soybeans produced (less weed material in the beans sold to crushers that resulted in price premia being obtained in the early years – no longer relevant post 2005), and cost savings derived.

- The average net increase in gross margin in 2006 was $59/ha (an average of $105/ha over the 8 years of commercial use: Table 6).
- At the national level, the increase in farm income amounted to $7.6 million in 2006. Cumulatively in the period 1999–2006, the increase in farm income was $44.6 million (in nominal terms).
- The yield gains in 2006 were equivalent to an 9% increase in national production (the annual average increase in production over the 8 years was equal to 10.1%).
- In added value terms, the combined effect of higher yields, improved quality of beans, and reduced cost of production on farm income in 2006 was equivalent to an annual increase in production of 9.3% (33,230 tons).

Mexico

GM HT soybeans were first planted commercially in Mexico in 1997 (on a trial basis) and in 2008, a continued trial area of 7,330 ha (out of total plantings of 88,000 ha) were varieties containing the GM HT trait.

At the farm level, the main impacts of use have been a combination of yield increase (+9.1% in 2004 and 2005, +3.64% in 2006, +3.2% 2007, and +2.4% 2008) and (herbicide) cost savings. The average farm income benefit has been within a range of $54–$89/ha (inclusive of yield gain, cost savings, and after payment of the technology fee/seed premium of $34.5/ha) and the increase in farm income at the national level was $0.04 million in 2008 (Table 7). The cumulative increase in farm income since 2004 has been $3.35 million (in nominal terms). In added value terms, the increase in farm income from the use of the GM HT technology in 2008 was equivalent to an annual increase in production of about 0.5%.

Bolivia

GM HT soybeans were officially permitted to be planted in 2008, although "illegal" plantings have occurred for several years. For the purposes of analysis

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 6 Farm-level income impact of using herbicide-tolerant soybeans in Romania 1999–2006

Year	Cost saving ($/ha)	Cost savings net of cost of technology ($/ha)	Net increase in gross margin ($/ha)	Impact on farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1999	162.08	2.08	105.18	1.63	4.0
2000	140.30	−19.7	89.14	3.21	8.2
2001	147.33	−0.67	107.17	1.93	10.3
2002	167.80	32.8	157.41	5.19	14.6
2003	206.70	76.7	219.01	8.76	12.7
2004	63.33	8.81	135.86	9.51	13.7
2005	64.54	9.10	76.16	6.69	12.2
2006	64.99	9.10	58.79	7.64	9.3

Sources and notes:

1. Impact data (Sources: Brookes [14] and Monsanto Romania [15]. Average yield increase 31% applied to all years to 2003 and reduced to +25% 2004, +19% 2005 and +13% 2006. Average improvement in price premia from high quality 2% applied to years 1999–2004
2. All values for prices and costs denominated in Romanian Lei have been converted to US dollars at the annual average exchange rate in each year
3. Technology cost includes cost of herbicides
4. The technology was not permitted to be planted from 2007 – due to Romania joining the EU

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 7 Farm-level income impact of using GM HT soybeans in Mexico 2004–2008

Year	Cost savings ($/ha)	Net cost saving/increase in gross margin (inclusive of technology cost and yield gain: $/ha)	Impact on farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
2004	49.44	82.34	1.18	3.07
2005	51.20	89.41	0.94	2.13
2006	51.20	72.98	0.51	1.05
2007	51.05	66.84	0.33	0.9
2008	33.05	54.13	0.40	0.5

Sources and notes:

1. Impact data based on Monsanto, 2005, 2007, and 2008 [16–18]. Reportes final del programa Soya Solución Faena en Chiapas. Monsanto Comercial
2. All values for prices and costs denominated in Mexican pesos have been converted to US dollars at the annual average exchange rate in each year

in this section, impacts have been calculated back to 2005, when an estimated 0.3 million hectares of soybeans used GM HT technology. In 2008, an estimated 453,000 ha (63% of total crop) used GM HT technology.

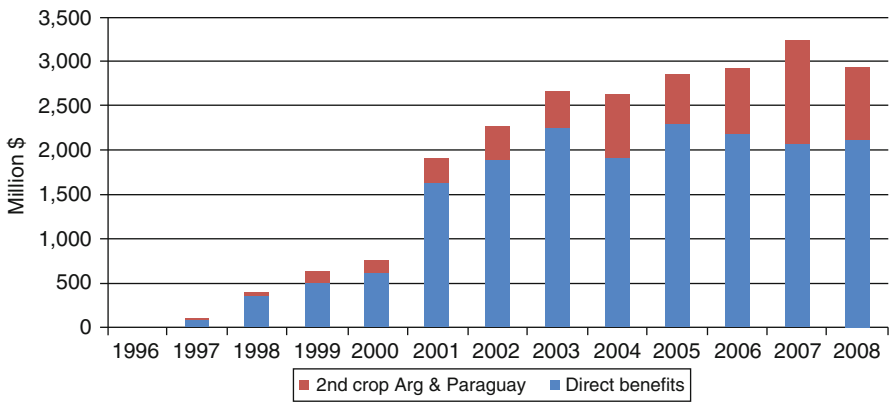
The main impacts of the technology are as follows (Table 8):

- An increase in yield arising from improved yield control. The research work conducted by

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 8 Farm-level income impact of using GM HT soybeans in Bolivia 2005–2008

Year	Cost savings excluding seed cost premium ($/ha)	Net cost saving/increase in gross margin (inclusive of technology cost and yield gain: $/ha)	Impact on farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
2005	9.28	39.73	12.08	4.09
2006	9.28	36.60	15.55	6.35
2007	9.28	44.40	19.45	7.37
2008	9.28	80.09	36.33	7.24

Sources and notes:
1. Impact data based on Fernandez et al. [19]. Average yield gain assumed +15%, cost of technology $3.32/ha



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 2
Global farm-level income benefits derived from using GM HT soybeans 1996–2008 (million $)

Fernandez et al. [19] estimated a 30% yield difference between GM HT and conventional soybeans although some of the yield gain reflected the use of poor-quality conventional seed by some farmers. In the analysis presented, a more conservative yield gain of +15% has been used.

- GM HT soybeans are assumed to trade at a price discount to conventional soybeans of −2.7%, reflecting the higher price set for conventional soybeans by the Bolivian government in 2008.
- The cost of the technology to farmers has been about $3.3/ha and the cost savings equal to about $9.3/ha, resulting in a net cost of production change of +$6/ha.
- Overall, in 2008, the average farm income gain from using GM HT soybeans was about $80/ha, resulting in a total farm income gain of $36.3 million. Cumulatively since 2005, the total farm income gain is estimated at $83.4 million.

Summary of Global Economic Impact

In global terms, the farm-level impact of using GM HT technology in soybeans was $2.12 billion in 2008 (Fig. 2). If the second crop benefits arising in Argentina are included, this impact rises to $2.92 billion. Cumulatively since 1996, the farm income benefit has been

(in nominal terms) $17.9 billion ($23.3 billion if second crop gains in Argentina and Paraguay are included).

In terms of the total value of soybean production from the countries growing GM HT soybeans in 2008, the additional farm income (inclusive of Argentine second crop gains) generated by the technology is equal to a value-added equivalent of 4.3%. Relative to the value of global soybean production in 2008, the farm income benefit added the equivalent of 4.1%.

These economic benefits should be placed within the context of a significant increase in the level of soybean production in the main GM adopting countries since 1996 (a 63% increase in the area planted in the leading soybean producing countries of the USA, Brazil, and Argentina).

These economic benefits mostly derive from cost savings although farmers in Mexico, Bolivia, and Romania also obtained yield gains (from significant improvements in weed control levels relative to levels applicable prior to the introduction of the technology). If it is also assumed that all of the second crop soybean gains are effectively additional production that would not have otherwise occurred without the GM HT technology (the GM HT technology facilitated major expansion of second crop soybeans in Argentina and to a lesser extent in Paraguay) then these gains are de facto "yield" gains. Under this assumption, of the total cumulative farm income gains from using GM HT soy, $5.56 billion (24%), is due to yield gains/second crop benefits and the balance, 76%, is due to cost savings.

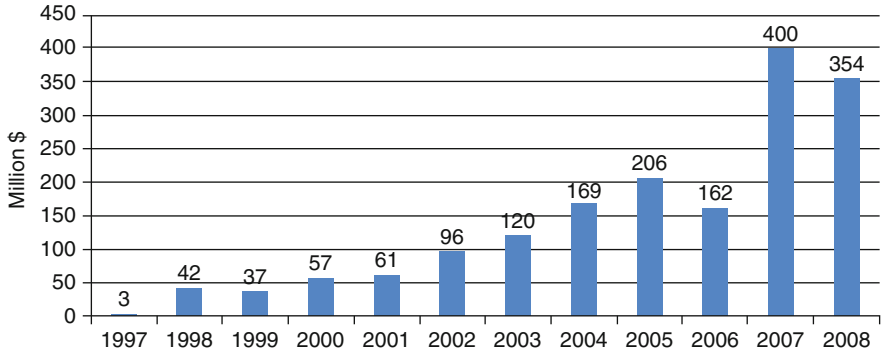
Herbicide-Tolerant Maize

The USA

Herbicide-tolerant maize has been used commercially in the USA since 1997 and in 2008 was planted on 63% of the total US maize crop. The impact of using this technology at the farm level is summarized in Fig. 3. As with herbicide-tolerant soybeans, the main benefit has been to reduce costs, and hence improve profitability levels. Average profitability improved by $20–$25/ha in most years ($17.6/ha in 2008 – affected by the significant increase in glyphosate prices relative to other herbicides). The net gain to farm income in 2008 was $354 million and cumulatively, since 1997 the farm income benefit has been $1.7 billion. In added value terms, the effect of reduced costs of production on farm income in 2008 was equivalent to an annual increase in production of 0.71% (2.17 million tons).

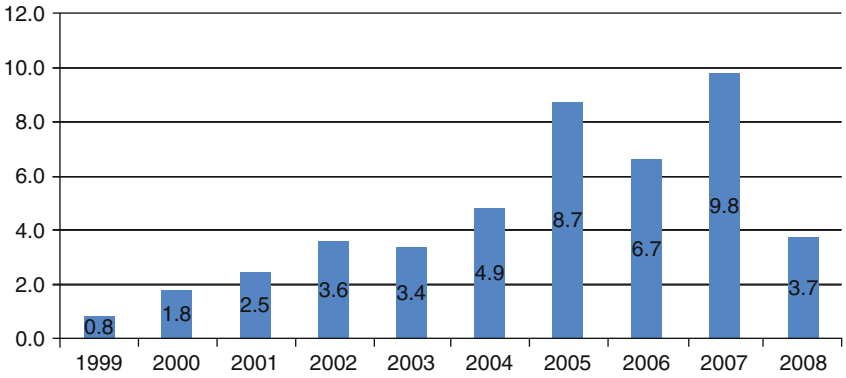
Canada

In Canada, GM HT maize was first planted commercially in 1997. By 2008, the proportion of total plantings accounted for by varieties containing a GM HT trait was 51%. As in the USA, the main benefit has been to reduce



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 3

National farm income impact of using GM HT maize in the USA 1997–2008 (Source and notes: Impact analysis based on Sankala and Blumenthal [6, 7] and Johnson and Strom [8] and updated for 2008 to reflect changes in herbicide prices. Estimated cost of the technology $14.83/ha in years up to 2004, $17.3/ha in 2005, $24.71/ha 2006 onward. Cost savings (mostly from lower herbicide use) $33.47/ha in 2004, $38.61/ha 2005, $29.27/ha 2006, $42.28/ha 2007, and $40.87/ha 2008)



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 4
National farm income impact of using GM HT maize in Canada 1999–2008 ($ million) (Source and notes: Impact analysis based on data supplied by Monsanto Canada. Estimated cost of the technology $18-$32/ha, cost savings (mostly from lower herbicide use) $31–$45/ha)

costs and to improve profitability levels. Average annual profitability has improved by between $12/ha and $18/ha up to 2007, but fell to about $6/ha in 2008 (due to the higher price increases for glyphosate relative to other herbicides). In 2008, the net increase in farm income was $3.7 million and cumulatively since 1999 the farm income benefit has been $45.8 million. In added value terms, the effect of reduced costs of production on farm income in 2008 was equivalent to an annual increase in production of 0.22% (23,500 tons: Fig. 4).

Argentina

GM HT maize was first planted commercially in Argentina in 2004 and in 2008, varieties containing a GM HT trait were planted on 805,000 ha (35% of the total maize area). It has been adopted in two distinct types of area, the majority (80%) in the traditional "corn production belt" and 20% in newer maize-growing regions, which have been traditionally known as more marginal areas that surround the "Corn Belt." The limited adoption of GM HT technology in Argentina up to 2006 was mainly due to the technology only being available as a single gene, not stacked with the GM IR trait, which most maize growers have also adopted. Hence, faced with an either GM HT or GM IR trait available for use, most farmers have chosen the GM IR trait because the additional returns derived from adoption have tended to be (on average) greater from the GM IR trait than the GM HT trait (see below for further details of returns from the GM HT trait). Stacked traits became available in 2007 and contributed to the significant increase in the GM HT maize area relative to 2006.

In relation to impact on farm income, the following observations were made:

- In all regions, the cost of the technology (about $20/ha) has been broadly equal to the saving in herbicide costs.
- In the Corn Belt area, use of the technology has resulted in an average 3% yield improvement via improved weed control. In the more marginal areas, the yield impact has been much more significant (+22%) as farmers have been able to significantly improve weed control levels.
- In 2008, the additional farm income at a national level from using GM HT technology has been +$61.6 million, and cumulatively since 2004, the income gain has been $113.8 million.

South Africa

Herbicide-tolerant maize has been grown commercially in South Africa since 2003, and 6,46,000 ha out of total plantings of 2.43 million hectares were herbicide tolerant in 2008. Farmers using the technology have found that small net savings in the cost of production have occurred (i.e., the cost saving from reduced expenditure on herbicides has been greater

than the cost of the technology), although in 2008, due to the significant rise in the global price of glyphosate relative to their herbicides, the net farm income balance was negative, at about −$2/ha. This resulted in a total net farm loss arising from using GM HT technology of $1.43 million, though since 2003, there has been a net cumulative income gain of $3.77 million.

Philippines

GM HT maize was first grown commercially in 2006, and 2008 was planted on 270,000 ha. Information about the impact of the technology is limited, although industry sources estimate that, on average farmers using it have derived a 15% increase in yield. Based on a cost of the technology of $24–$27/ha (and assuming no net cost savings), the net national impact on farm income was +$15.9 million in 2008. Cumulatively, since 2006, the total farm income gain has been $27.1 million

Summary of Global Economic Impact

In global terms, the farm-level economic impact of using GM HT technology in maize was $433.5 million in 2008 (82% of which was in the USA). Cumulatively since 1997, the farm income benefit has been (in nominal terms) $1.9 billion. Of this, 92% has been due to cost savings and 8% to yield gains (from improved weed control relative to the level of weed control achieved by farmers using conventional technology).

In terms of the total value of maize production in the main countries using this technology in 2008, the additional farm income generated by the technology is equal to a value-added equivalent of 0.3% of global maize production.

Herbicide-Tolerant Cotton

The USA

GM HT cotton was first grown commercially in the USA in 1997 and in 2008 was planted on 68% of total cotton plantings.

The farm income impact of using GM HT cotton is summarized in Table 9. The primary benefit has been to reduce costs, and hence improve profitability levels, with annual average profitability increasing by between $21/ha and $49/ha (the only published source that has examined the impact of HT cotton in the USA is the work by Sankala and Blumenthal [6, 7], and Johnson and Strom [8]. In the 2001 study, the costs saved were based on historic patterns of herbicides used on conventional cotton in the mid/late 1990s. The latter studies estimated cost savings on the basis of the conventional herbicide treatment that would be required to deliver the same level of weed control as GM HT cotton. Revised analysis has, however, been conducted for 2008 to reflect changes in the costs of production (notably cost of the technology (in particular "Roundup Ready Flex technology"), higher prices for glyphosate relative to other herbicides in 2008 and additional costs incurred to control weeds resistant to glyphosate in some regions) in the years up to 2004. Since then, net income gains have fallen to between $1/ha and $5/ha. The relatively small positive impact on direct farm income in 2008 (and in the last few years) reflects a combination of reasons, including the higher cost of the technology, significant price increases for glyphosate relative to price increases for other herbicides, and additional costs incurred for management of weeds resistant to glyphosate (notably *Palmer Amaranth*). Overall, the net direct farm income impact in 2008 is estimated to be $2.5 million (this does not take into consideration any nonpecuniary benefits associated with adoption of the technology: see Section 3.9). Cumulatively, since 1997, there has been a net farm income benefit from using the technology of $799 million.

Other Countries

Australia, Argentina, South Africa, and Mexico are the other countries where GM HT cotton is commercially grown; from 2000 in Australia, 2001 in South Africa, 2002 in Argentina, and 2005 in Mexico. In 2008, 79% (50,460 ha), 38% (124,000 ha), 75% (9,750 ha), and 40% (50,000 ha) respectively of the total Australian, Argentine, South African, and Mexican cotton crops were planted to GM HT cultivars.

We are not aware of any published research into the impact of GM HT cotton in South Africa, Argentina, or Mexico. In Australia, although research has been conducted into the impact of using GM HT cotton (e.g., Doyle et al. [20]) this does not provide

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 9 Farm-level income impact of using GM HT cotton in the USA 1997–2008

Year	Cost savings ($/ha)	Net cost saving/increase in gross margins, inclusive of cost of technology ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1997	34.12	21.28	12.56	0.2
1998	34.12	21.28	30.21	0.58
1999	34.12	21.28	53.91	1.29
2000	34.12	21.28	61.46	1.22
2001	65.59	45.27	161.46	4.75
2002	65.59	45.27	153.18	3.49
2003	65.59	45.27	129.75	2.33
2004	83.35	48.80	154.72	2.87
2005	71.12	2.89	9.57	0.18
2006	73.66	3.31	13.29	0.22
2007	76.01	5.40	16.56	0.32
2008	72.76	1.20	2.50	0.08

Source and notes:

1. Impact analysis based on Sankala and Blumenthal [6, 7] and Johnson and Strom [8] and own analysis for 2008
2. Estimated cost of the technology $12.85/ha (1997–2000) and $21.32/ha 2001–2003, $34.55 2004, $68.22/ha 2005, $70.35/ha 2006, $70.61/ha 2007, and £71.56/ha 2008

quantification of the impact. Drawing on industry source estimates, the main impacts are as follows:

- *Australia*: No yield gain and cost of the technology in the range of $30–$45/ha up to 2007. The cost of the technology increased with the availability of "Roundup Ready Flex" and in 2008 was about $63/ha. The cost savings from the technology (after taking into consideration the cost of the technology have delivered small net gains of $5–$7/ha, although estimates relating to the net average benefits from Roundup Ready Flex are about $25/ha in 2008 [20]. Overall, in 2008, the total farm income from using the technology was about $3 million and cumulatively, since 2000, the total gains have been $8.3 million.
- *Argentina*: No yield gain and a cost of technology in the range of $30–$40/ha, although with the increasing availability of stacked traits in recent years, the "cost" part of the HT technology has fallen to $24/ha. Net farm income gains (after deduction of the cost of the technology) have been $8–$18/ha and in 2008 were just under $10/ha. Overall, in 2008, the total farm income from using GM HT cotton technology was about $7.4 million, and cumulatively since 2002, the farm income gain has been $34.2 million.
- *South Africa*: No yield gain and a cost of technology in the range of $15–$25/ha. Net farm income gains from cost savings (after deduction of the cost of the technology) have been $30–$60/ha. In 2008, the average net gain was $33.6/ha and the total farm income benefit of the technology was $0.37 million. Cumulatively since 2001, the total farm income gain from GM HT cotton has been $2.2 million.
- *Mexico*: Average yield gains of +3.6% from improved weed control have been reported in the first 3 years of use, although no yield gain was recorded in 2008. The average cost of the technology has been in the range of $60–$66/ha and typical net farm income gains of about $80/ha, though in 2008, with no yield gains this fell back to $16/ha.

Overall, in 2008, the total farm income gain from using GM HT cotton was about $1.35 million and cumulatively since 2005, the total farm income gain has been $11.7 million.

Summary of Global Economic Impact

Across the five countries using GM HT cotton in 2008, the total farm income impact derived from using GM HT cotton was +$14.6 million. Cumulatively since 1997, there have been net farm income gains of $855.8 million (93% of this benefit has been in the USA). Of this, 96% has been due to cost savings and 4% to yield gains (from improved weed control relative to the level of weed control achieved using conventional technology).

Herbicide-Tolerant Canola

Canada

Canada was the first country to commercially use GM HT canola in 1996. Since then, the area planted to varieties containing GM HT traits has increased significantly, and in 2008 was 83% of the total crop (5.43 million hectares).

The farm-level impact of using GM HT canola in Canada since 1996 is summarized in Table 10. The key features are as follows:

- The primary impact in the early years of adoption was increased yields of almost 11% (e.g., in 2002 this yield increase was equivalent to an increase in total Canadian canola production of nearly 7%). In addition, a small additional price premia was achieved from crushers through supplying cleaner crops (lower levels of weed impurities). With the development of hybrid varieties using conventional technology, the yield advantage of GM HT canola relative to conventional alternatives (the main one of which is "Clearfield" conventionally derived herbicide-tolerant varieties. Also, hybrid canolas now account for the majority of plantings (including some GM hybrids) with the hybrid vigor delivered by conventional breeding techniques (even in the GM HT [to glyphosate] varieties) has been eroded. As a result, our analysis has applied the yield advantage of +10.7% associated with the GM HT technology in its early years of adoption (source: Canaola Council study of 2001) to 2003. From 2004, the yield gain has been based on differences between average annual variety trial results for "Clearfield" (conventional herbicide-tolerant varieties) and biotech alternatives. The biotech alternatives have also been differentiated into glyphosate tolerant and glufosinate tolerant. This resulted in the following observation: for GM glyphosate-tolerant varieties no yield difference for 2004, 2005, and 2008 and +4% 2006 and 2007. For GM glufosinate-tolerant varieties, the yield differences were +12% 2004 and 2008, +19% 2005, +10% 2006 and 2007. The quality premia associated with cleaner crops (see above) has not been included in the analysis from 2004.
- Cost of production (excluding the cost of the technology) has fallen, mainly through reduced expenditure on herbicides and some savings in fuel and labor. These savings have annually been between about $25/ha and $36/ha. The cost of the technology to 2003 was however marginally higher than these savings resulting in a net increase in costs of $3– $5/ha. On the basis of comparing GM HT canola with "Clearfield" HT canola (from 2004), there has been a net cost saving of between $5/ha and $10/ha, although in 2008 this was $17/ha.
- The overall impact on profitability (inclusive of yield improvements and higher quality) has been an increase of between $22/ha and $48/ha up to 2003. On the basis of comparing GM HT canola with "Clearfield" HT canola (from 2004), the net increase in profitability has been between $23/ha and $66/ha.
- The annual total national farm income benefit from using the technology has risen from $6 million in 1996 to $364 million in 2008. The cumulative farm income benefit over the 1996–2008 period (in nominal terms) was $1.64 billion.
- In added value terms, the increase in farm income in 2008 has been equivalent to an annual increase in production of 6.3%.

The USA

GM HT canola has been planted on a commercial basis in the USA since 1999. In 2008, 95% of the US canola crop was GM HT (380,230 ha).

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 10 Farm-level income impact of using GM HT canola in Canada 1996–2008

Year	Cost savings ($/ha)	Cost savings inclusive of cost of technology ($/ha)	Net cost saving/ increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1996	28.59	−4.13	45.11	6.23	0.4
1997	28.08	−4.05	37.11	21.69	1.17
1998	26.21	−3.78	36.93	70.18	3.43
1999	26.32	−3.79	30.63	90.33	5.09
2000	26.32	−3.79	22.42	59.91	5.08
2001	25.15	−1.62	23.10	53.34	5.69
2002	24.84	−3.59	29.63	61.86	6.17
2003	28.04	−4.05	41.42	132.08	6.69
2004	21.42	+4.44	19.09	70.72	4.48
2005	23.11	+4.50	32.90	148.12	6.56
2006	34.02	+16.93	50.71	233.13	8.09
2007	35.44	+17.46	66.39	341.44	7.54
2008	36.36	+17.56	66.63	364.23	6.35

Sources and notes:

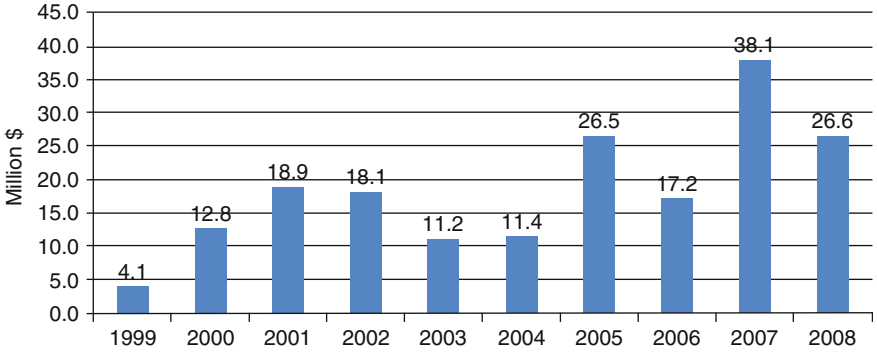
1. Impact data based on Canola Council study [21] to 2003 and Gusta et al. [22]. Includes a 10.7% yield improvement and a 1.27% increase in the price premium earned (cleaner crop with lower levels of weed impurities) until 2003. After 2004, the yield gain has been based on differences between average annual variety trial results for Clearfield and biotech alternatives. The biotech alternatives have also been differentiated into glyphosate tolerant and glufosinate tolerant. This resulted in the following observation: for GM glyphosate-tolerant varieties no yield difference for 2004, 2005, and 2008 and +4% 2006 and 2007. For GM glufosinate-tolerant varieties, the yield differences were +12% 2004 and 2008, +19% 2005, +10% 2006 and 2007

2. Negative values denote a net increase in the cost of production (i.e., the cost of the technology was greater than the other cost (e.g., on herbicides) reductions

3. All values for prices and costs denominated in Canadian dollars have been converted to US dollars at the annual average exchange rate in each year

The farm-level impact has been similar to the impact identified in Canada. More specifically, the following observations were noted:

- Average yields increased by about 6% in the initial years of adoption. As in Canada (see above) the availability of high-yielding hybrid conventional varieties has eroded some of this yield gain in recent year relative to conventional alternatives. As a result, the positive yield impacts post 2004 have been applied on the same basis as in Canada (comparison with Clearfields: see Canada above).
- The cost of the technology has been $12–$17/ha for glufosinate-tolerant varieties and $12–$33/ha for glyphosate-tolerant varieties. Cost savings (before inclusion of the technology costs) have been $35–$45/ha ($22/ha in 2008) for glufosinate-tolerant canola and $40–$79/ha for glyphosate-tolerant canola.
- The net impact on gross margins has been between +$22/ha and +$90/ha ($5/ha in 2008) for glufosinate-tolerant canola, and +$28/ha and +$61/ha for glyphosate-tolerant canola.
- At the national level, the total farm income benefit in 2008 was $26.6 million (Fig. 5) and the cumulative benefit since 1999 has been $185 million.
- In added value terms, the increase in farm income in 2008 has been equivalent to an annual increase in production of about 10.3%.



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 5
National farm income impact of using GM HT canola in the USA 1999–2008 (Source and notes: Impact analysis based on Sankala and Blumenthal [6, 7] and Johnson and Strom [8]. Decrease in total farm income impact 2002–2004 is due to decline in total plantings of canola in the USA (from 612,000 in 2002 to 316,000 ha in 2004). Positive yield impact applied in the same way as Canada from 2004)

Australia

GM HT canola was permitted for commercial use in the two states of Victoria and New South Wales in 2008, and was planted on 10,100 ha in that year (2008/09). Ninety-five percent of these plantings had tolerance to the herbicide glyphosate and the balance were tolerant to glufosinate.

A fairly comprehensive farm survey-based analysis of impact of the glyphosate-tolerant canola was commissioned by Monsanto, which involved interviews with 92 of the 108 farmers using this technology in 2008/09 [23, 24]. Key findings from this survey are as follows:

- The technology was made available in both open-pollinated and hybrid varieties, with the open-pollinated varieties representing the cheaper end of the seed market, where competition was mainly with open-pollinated varieties containing herbicide tolerance (derived conventionally) to herbicides in the triazine (TT) group. The hybrid varieties containing glyphosate tolerance competed with nonherbicide-tolerant conventional hybrid varieties and herbicide-tolerant "Clearfield" hybrids (tolerant to the imidazolinone group of herbicides), although, were used in 2008, all of the 33 farmers in the survey using GM HT hybrids did so mainly in competition and comparison with "Clearfield" varieties.
- The GM HT open-pollinated varieties sold to farmers at a premium of about $Aus3/ha (about $2.5 US/ha) relative to the TT varieties. The GM HT hybrids sold at a seed premium of about $Aus 9/ha ($7.55 US/ha) compared to "Clearfield" hybrids. In addition, farmers using the GM HT technology paid a "technology" fee in two parts; one part was a set fee of $Aus500 per farm plus $Aus 10.2/ton of output of canola. On the basis that there were 108 farmers using GM HT (glyphosate tolerant) technology in 2008, the average "up front" fee paid for the technology was $Aus5.62/ha. On the basis of average yields obtained for the two main types of GM HT seed used, those using open-pollinated varieties paid $11.83/ha (basis: average yield of 1.16 tons/ha) and those using GM HT hybrids paid $Aus12.95/ha (basis: average yield of 1.27 tons/ha). Therefore, the total seed premium and technology fee paid by farmers for the GM HT technology in 2008–2009 was $Aus20.45/ha ($17.16 US/ha) for open-pollinated varieties and $Aus 27.57/ha ($23.13 US/ha) for hybrid varieties. After taking into consideration, the seed premium/technology fees, the GM HT system was marginally more expensive by $Aus3/ha ($2.5 US/ha) and $Aus4/ha ($US 3.36/ha) respectively for weed control than the TT and Clearfield varieties.
- The GM HT varieties delivered higher average yields than their conventional counterparts: +22.11% compared to the TT varieties and +4.96% compared to the "Clearfield" varieties.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 11 Farm-level income impact of using GM HT canola in Australia 2008 ($US)

Year	Average cost saving ($/ha)	Average cost savings (net after cost of technology ($/ha)	Average net increase in gross margins ($/ha)	Increase in farm income at a national level ($)
2008	19.18	−20.77	93.37	943,054

Source derived from and based on Monsanto survey of license holders 2008

Notes:

1. The average values shown are weighted averages
2. Other weighted average values derive include yield +21.1% and quality (price) premium of 2.1% applied on the basis of this level of increase in average oil content

In addition, the GM HT varieties produced higher oil contents of +2% and +1.8% respectively compared to TT and "Clearfield" varieties.

- The average reduction in weed control costs from using the GM HT system (excluding seed premium/technology fee) was $Aus 17/ha for open-pollinated varieties (competing with TT varieties) and $Aus 24/ha for hybrids (competing with Clearfield varieties).

In the analysis summarized below in Table 11, these research findings have been applied to the total GM HT crop area on a weighted basis in which the results of GM HT open-pollinated varieties that compete with TT varieties were applied to 64% of the total area and the balance of area used the results from the GM HT hybrids competing with "Clearfield" varieties. This weighting reflects the distribution of farms in the survey, in which 59 (64%) of the farmers indicated they grew open-pollinated varieties and 33 (34%) grew hybrids. The findings show an average farm income gain of $US 93/ha and a total farm income gain of $0.93 million in 2008.

Summary of Global Economic Impact

In global terms, the farm-level impact of using GM HT technology in canola in Canada, the USA, and Australia was $392 million in 2008. Cumulatively, since 1996, the farm income benefit has been (in nominal terms) $1.83 billion. Within this, 79% has been due to yield gains and the balance (21%) has been from cost savings.

In terms of the total value of canola production in these three countries in 2008, the additional farm income generated by the technology is equal to a value-added equivalent of 6.9%. Relative to the value of global canola production in 2008, the farm income benefit added the equivalent of 1.5%.

GM Herbicide-Tolerant (GM HT) Sugar Beet

GM HT sugar beet was first grown commercially in the USA in 2007 (under 1,000 ha), although it was 2008 before sufficient quantities of seed were available for widespread commercial cultivation. In 2008, just under 258,000 ha of GM HT sugar beet were planted, equal to about 63.5% of the total US crop. The highest levels of penetration of the technology (85% plus of total crop) occurred in Idaho, Wyoming, Nebraska, and Colorado, with about 50% of the crops in the largest sugar beet growing states of North Dakota and Michigan being GM HT.

Impact of the technology in these early years of adoption has been identified as follows:

(a) *Yield*: Analysis by Kniss [25] covering a limited number of farms in Wyoming (2007) identified positive yield impacts of +8.8% in terms of additional root yield (from better weed control) and +12.6% in terms of sugar content relative to conventional crops (i.e., the GM HT crop had about a 3.8% higher sugar content, which amounts to a 12.8% total sucrose gain relative to conventional sugar beet once the root yield gain was taken into consideration). In contrast, Khan [26] found similar yields reported between conventional and GM HT sugar beet in the Red River Valley region (North Dakota) and Michigan. These contrasting results probably reflect a combination of factors including:

- The sugar beet growing regions in Wyoming can probably be classified as high weed problem areas, and as such, are regions where obtaining effective weed control is difficult using conventional technology (timing of application is key to weed control in sugar beet, with optimal time for application being when weeds are small). Also some weeds (e.g., Kochia) are

resistant to some of the commonly used ALS inhibitor herbicides like chlorsulfuron. The availability of GM HT sugar beet with its greater flexibility on application timing has therefore potentially delivered important yield gains for such growers.

- The GM HT trait was not available in all leading varieties suitable in all growing regions in 2008, hence the yield benefits referred to above from better weed control have to some extent been counterbalanced by only being available in poorer performing germ plasm in states like Michigan and North Dakota (notably not being available in 2008 in leading varieties with rhizomania resistance). It should be noted that the authors of the research cited in this section both perceive that yield benefits from using GM HT sugar beet will be a common feature of the technology in most regions once the technology is available in leading varieties.
- The year 2008 was reported to have been, in the leading sugar beet growing states, a reasonable year for controlling weeds through conventional technology (i.e., it was possible to get good levels of weed control through timely applications), hence the similar performance reported between the two systems.

(b) Costs of production

- Kniss's work in Wyoming identified weed control costs (comprising herbicides, application, cultivation, and hand labor) for conventional beet of $437/ha compared to $84/ha for the GM HT system. After taking into consideration the $131/ha seed premium/technology fee for the GM HT trait, the net cost differences between the two systems was $222/ha in favor of the GM HT system. Kniss did, however, acknowledge that the conventional costs associated with this sample were high relative to most producers (reflecting application of maximum dose rates for herbicides and use of hand labor), with a more typical range of conventional weed control costs being between $171/ha and $319/ha (average $245/ha).
- Khan's analysis puts the typical weed control costs in the Red River region of North Dakota to be about $227/ha for conventional compared to $91/ha for GM HT sugar beet. After taking into consideration the seed premium/technology fee (assumed by Khan to be $158/ha), the total weed control costs were $249/ha for the GM HT system, $22/ha higher than the conventional system. Despite this net increase in average costs of production, most growers in this region used (and planned to continue using), the GM HT system because of the convenience and weed control flexibility benefits associated with it (which research by Marra and Piggot [1]) estimated in the corn, soybean, and cotton sectors to be valued at between $12/ha and $25/ha to US farmers). It is also likely that Khan's analysis may understate the total cost savings from using the technology by not taking into account savings on application costs and labor for hand weeding.

For the purposes of our analysis, we have drawn on both these pieces of work, as summarized in Table 12. This shows a net farm income gain in 2008 of over $21 million to US sugar beet farmers (average gain per hectare of just under $83/ha). With the availability of GM HT technology in more of the leading varieties, it is expected that the farm income gains associated with yield gains will be greater in subsequent years.

GM Insect-Resistant (To Corn-Boring Pests: GM IR) Maize

The USA

GM IR maize was first planted in the USA in 1996 and in 2008, seed containing GM IR traits was planted on 57% (18.14 million hectares) of the total US maize crop.

The farm-level impact of using GM IR maize in the USA since 1996 is summarized in Table 13:

- The primary impact has been increased average yields of about 5% (in 2008 this additional production is equal to an increase in total US maize production of +2.41%).
- The net impact on cost of production has been a small increase of between $1/ha and $9/ha (additional cost of the technology being higher than the estimated average insecticide cost savings of $15–$16/ha).
- The annual total national farm income benefit from using the technology has risen from $8.76 million in

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 12 Farm-level income impact of using GM HT sugar beet in the USA 2007–2008

Year	Average cost saving ($/ha)	Average cost savings (net after cost of technology ($/ha)	Average net increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
2007	353.35	222.39	584.00	472,680	0.03
2008	142.50	−8.58	82.88	21,380,290	1.83

Sources derived from and based on Kniss [25] and Khan [26]

Notes:

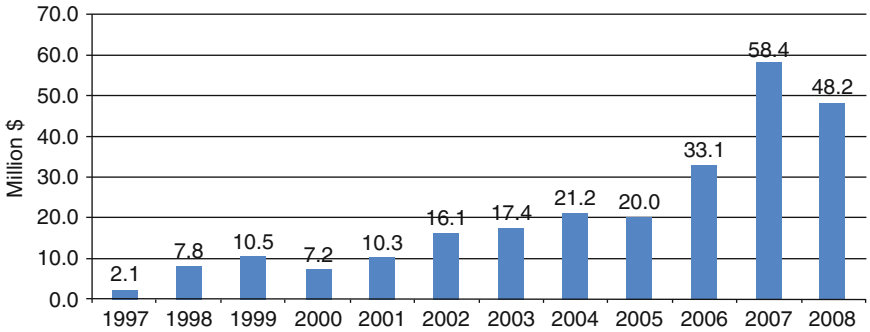
1. The yield gains identified by Kniss have been applied to the 2007 GM HT plantings in total and to the estimated GM HT plantings in the states of Idaho, Wyoming, Nebraska, and Colorado, where penetration of plantings in 2008 was 85% (these states account for 26% of the total GM HT crop in 2008), and which are perceived to be regions of above average weed problems. For all other regions, no yield gain is assumed. Across the entire GM HT area in 2008, this equates to a net average yield gain of +3.28%
2. The seed premium of $131/ha, average costs of weed control respectively for conventional and GM HT systems of $245/ha and $84/ha, from Kniss were applied to the crop in Idaho, Wyoming, Nebraska, and Colorado. The seed premium of $158/ha, weed control costs of $227/ha and $249/ha respectively for conventional and GM HT sugar beet, identified by Khan were applied to all other regions using the technology. These states account for 26% of the total GM HT crop in 2008. The resulting average values for seed premium/cost of technology across the entire 2008 GM HT crop was therefore $151.08/ha and the average weed control cost saving associated with the GM HT system (before taking into consideration the seed premium) was $142.5/ha

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 13 Farm-level income impact of using GM IR maize in the USA 1996–2008

Year	Cost saving ($/ha)	Cost savings (net after cost of technology ($/ha)	Net increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1996	24.71	−9.21	29.20	8.76	0.03
1997	24.71	−9.21	28.81	70.47	0.27
1998	20.30	−4.8	27.04	167.58	0.77
1999	20.30	−4.8	25.51	206.94	1.04
2000	22.24	−6.74	24.32	148.77	0.71
2001	22.24	−6.74	26.76	155.87	0.72
2002	22.24	−6.74	30.74	240.45	0.96
2003	22.24	−6.74	31.54	291.00	1.14
2004	15.88	−6.36	33.82	363.41	1.32
2005	15.88	−1.42	34.52	399.91	1.60
2006	15.88	−1.42	55.78	707.23	1.86
2007	15.88	−1.42	61.22	1,136.21	2.28
2008	24.71	−8.83	67.51	1,224.59	2.40

Sources and notes:

1. Impact data based on a combination of studies including the ISAAA (James) review [27], Marra et al. [3], Sankala and Blumenthal [6, 7], and Johnson and Strom [8], Gianessi and Carpenter [28]
2. Yield impact +5% based on average of findings of above studies
3. Insecticide cost savings based on the above references
4. – (minus) value for net cost savings means the cost of the technology is greater than the other cost savings



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 6

National farm income impact of using GM IR maize in Canada 1996–2008

Notes:

1. Yield increase of 5% based on industry assessments (consistent with US analysis). Cost of technology and insecticide cost savings based on US analysis,

2. GM IR area planted in 1996 = 1,000 ha,

3. All values for prices and costs denominated in Canadian dollars have been converted to US dollars at the annual average exchange rate in each year

1996 to $1.22 billion in 2008. The cumulative farm income benefit over the 1996–2008 period (in nominal terms) was $5.12 billion.

- In added value terms, the increase in farm income in 2008 was equivalent to an annual increase in production of 2.4%.

Canada

GM IR maize has also been grown commercially in Canada since 1996. In 2008, it accounted for 62% of the total Canadian maize crop of 1.2 million hectares. The impact of GM IR maize in Canada has been very similar to the impact in the USA (similar yield and cost of production impacts). At the national level, in 2008 the additional farm income generated from the use of GM IR maize was $48.2 million and cumulatively since 1996 the additional farm income (in nominal terms) was $252 million (Fig. 6).

Argentina

In 2008, GM IR maize traits were planted on 75% of the total Argentine maize crop (GM IR varieties were first planted in 1998).

The main impact of using the technology on farm profitability has been via yield increases. Various studies (e.g., see ISAAA review in James [27]) and Trigo and Cap [29] have identified an average yield increase in the region of 8–10%, hence an average of 9% has been used in the analysis up to 2004. More recent trade source estimates provided to the authors put the average yield increased in the last 2–3 years to be between 5% and 6%. Accordingly, our analysis uses a yield increase value of 5.5% for the years from 2004.

No savings in costs of production have arisen for most farmers because very few maize growers in Argentina have traditionally used insecticides as a method of control for corn-boring pests. As such, average costs of production have increased by $20–$22/ha (the cost of the technology).

The net impact on farm profit margins (inclusive of the yield gain) has, in recent years, been an increase of about $20/ha. In 2008, the national level impact on profitability was an increase of $41 million (an added value equal to 2.15% of the total value of production). Cumulatively, the farm income gain since 1997 has been $269.7 million.

South Africa

GM IR maize has been grown commercially in South Africa since 2000. In 2008, 56% of the country's total maize crop of 2.42 million hectares used GM IR cultivars.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 14 Farm-level income impact of using GM IR maize in South Africa 2000–2008

Year	Cost savings ($/ha)	Net cost savings inclusive of cost of technology ($/ha)	Net increase in gross margin ($/ha)	Impact on farm income at a national level ($ millions)
2000	13.98	1.87	43.77	3.31
2001	11.27	1.51	34.60	4.46
2002	8.37	0.6	113.98	19.35
2003	12.82	0.4	63.72	14.66
2004	14.73	0.46	20.76	8.43
2005	15.25	0.47	48.66	19.03
2006	14.32	−2.36	63.75	63.05
2007	13.90	0.22	182.90	225.70
2008	11.74	−4.55	87.07	117.73

Sources and notes:

1. Impact data (Sources: Gouse [30–32] and Van der Weld [33])
2. Negative value for the net cost savings = a net increase in costs (i.e., the extra cost of the technology was greater than the other (e.g., less expenditure on insecticides) cost savings
3. All values for prices and costs denominated in South African Rand have been converted to US dollars at the annual average exchange rate in each year

The impact on farm profitability is summarized in Table 14. The main impact has been an average yield improvement of between 5% and 32% in the years 2000–2004, with an average of about 15% (used as the basis for analysis 2005–2007). In 2008, the estimated yield impact was +10.6% (source: Van der Weld [33]). The cost of the technology $8–$17/ha has broadly been equal to the average cost savings from no longer applying insecticides to control corn-boring pests.

At the national level, the increase in farm income in 2008 was $117.7 million and cumulatively since 2000 it has been $476 million. In terms of national maize production, the use of GM IR technology on 56% of the planted area has resulted in a net increase in national maize production of 5.9% in 2008. The value of the additional income generated was also equivalent to an annual increase in production of about 5.1%.

Spain

Spain has been commercially growing GM IR maize since 1998 and in 2008, 22% (79,270 ha) of the country's maize crop was planted to varieties containing a GM IR trait.

As in the other countries planting GM IR maize, the main impact on farm profitability has been increased yields (an average increase in yield of 6.3% across farms using the technology in the early years of adoption). With the availability and widespread adoption of the Mon 810 trait from 2003, the reported average positive yield impact is about +10%. There has also been a net annual average saving on cost of production (from lower insecticide use) of between $37/ha and $61/ha (Table 15). At the national level, these yield gains and cost savings have resulted in farm income being boosted, in 2008 by $17.9 million and cumulatively since 1998 the increase in farm income (in nominal terms) has been $77.9 million.

Relative to national maize production, the yield increases derived from GM IR maize were equivalent to a 2.2% increase in national production (2008). The value of the additional income generated from Bt maize was also equivalent to an annual increase in production of 2.1%.

Other EU countries

A summary of the impact of GM IR technology in other countries of the EU is presented in Table 16. This shows

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 15 Farm-level income impact of using GM IR maize in Spain 1998–2008

Year	Cost savings ($/ha)	Net cost savings inclusive of cost of technology ($/ha)	Net increase in gross margin ($/ha)	Impact on farm income at a national level ($ millions)
1998	37.40	3.71	95.16	2.14
1999	44.81	12.80	102.20	2.56
2000	38.81	12.94	89.47	2.24
2001	37.63	21.05	95.63	1.10
2002	39.64	22.18	100.65	2.10
2003	47.50	26.58	121.68	3.93
2004	51.45	28.79	111.93	6.52
2005	52.33	8.72	144.74	7.70
2006	52.70	8.78	204.5	10.97
2007	57.30	9.55	274.59	20.63
2008	61.49	10.25	225.36	17.86

Sources and notes:

1. Impact data (based on Brookes [34] and Brookes [35]). Yield impact +6.3% to 2004 and 10% used thereafter (originally Bt 176, latterly Mon 810). Cost of technology based on €18.5/ha to 2004 and €35/ha from 2005
2. All values for prices and costs denominated in Euros have been converted to US dollars at the annual average exchange rate in each year

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 16 Farm-level income impact of using GM IR maize in other EU countries 2005–2008

	Year first planted GM IR maize	Area 2008 (hectares)	Yield impact (%)	Cost of technology 2008 ($/ha)	Cost savings 2008 (before deduction of cost of technology: $/ha)	Net increase in gross margin 2008 ($/ha)	Impact on farm income at a national level 2008 (million $)
France	2005	Nil	N/p	N/p	N/p	N/p	N/p
Germany	2005	3,173	+4	58.57	73.21	78.64	0.25
Portugal	2005	4,851	+12.5	51.24	0	75.60	0.37
Czech Republic	2005	8,380	+10	51.24	26.35	101.95	0.85
Slovakia	2005	1,930	+12.3	51.24	0	228.31	0.44
Poland	2006	3,000	+12.5	51.24	0	133.08	0.40
Romania	2007	7,146	+7.1	46.85	0	26.59	0.19
Total other EU (excluding Spain)		28,480					2.5

Source and notes:

1. Source: Based on Brookes [35]
2. All values for prices and costs denominated in Euros have been converted to US dollars at the annual average exchange rate in each year
3. N/p – planting not permitted in France in 2008

that in 2008, the additional farm income derived from using GM IR technology in these six countries was +$2.5 million, and cumulatively over the 2005–2008 period, the total income gain was $11.1 million.

Other Countries

GM IR maize has been grown commercially in the following countries:

- *The Philippines* since 2003. In 2008, 280,000 ha out of total plantings of 2.6 million (7%) were GM IR. Estimates of the impact of using GM IR (Sources: Gonsalves [36], Yorobe [37], and Ramon [38]) show annual average yield increases in the range of 14.3–34%. Taking the midpoint of this range (+24.15%), coupled with a small average annual insecticide cost saving of about $12–$13/ha and average cost of the technology of about $33/ha, the net impact on farm profitability has been between $37/ha and $109/ha. In 2008, the national farm income benefit derived from using the technology was $33.5 million and cumulative farm income gain since 2003 has been $61.2 million.
- *Uruguay* since 2004, and in 2008, 110,000 ha (73% of the total crop) were GM IR. Using Argentine data as the basis for assessing impact, the cumulative farm income gain over the 3 years has been $3.9 million.
- *Brazil* starting in 2008, when 1.45 million hectares were planted to varieties containing a GM IR trait. Based on analysis from Galveo [12], the average yield impact was +4.66%, the cost of the technology was $21.6/ha, insecticide cost savings were $42/ha, and the average improvement to farm income equal to $48.12/ha. Overall, the increase in farm profitability associated with the adoption in 2008 was $69.8 million;
- *Honduras*. Here farm-level "trials" have been permitted since 2003, and in 2008, an estimated 9,000 ha used GM IR traits. Evidence from Falck Zepeda et al. [39] indicated that the primary impact of the technology has been to increase average yields (in 2008 +24%). As insecticides have not traditionally been used by most farmers, no costs of production savings have arisen, coupled with no additional cost for use of the technology (which has been provided free of charge for the trials). In our analysis, we have, however assumed a cost of the technology of $30/ha, and based on this, the estimated farm income benefit derived from the technology was $1.1 million in 2008 and cumulatively since 2003 the income gain has been $2 million.

Summary of Economic Impact

In global terms, the farm-level impact of using GM IR maize was $1.56 billion in 2008. Cumulatively since 1996, the benefit has been (in nominal terms) $6.34 billion. This farm income gain has mostly derived from improved yields (less pest damage) although in some countries farmers have derived a net cost saving associated with reduced expenditure on insecticides.

In terms of the total value of maize production from the countries growing GM IR maize in 2008, the additional farm income generated by the technology is equal to a value-added equivalent of 2.2%. Relative to the value of global maize production in 2008, the farm income benefit added the equivalent of 1.2%.

Insect-Resistant (Bt) Cotton (GM IR)

The USA

GM IR cotton has been grown commercially in the USA since 1996 and by 2008, was used in 63% (1.93 million hectares) of total cotton plantings.

The farm income impact of using GM IR cotton is summarized in Table 17. The primary benefit has been increased yields (by 9–11%), although small net savings in costs of production have also been obtained (reduced expenditure on insecticides being marginally greater than the cost of the technology). Overall, average profitability levels increased by $53–$115/ha with Bollgard I cotton (with a single Bt gene) between 1996 and 2002 and by between $87/ha and $118/ha in 2003–2008 with Bollgard II (containing two Bt genes and offering a broader spectrum of control). This resulted in a net gain to farm income in 2008 of $189 million. Cumulatively, since 1996, the farm income benefit has been $2.44 billion. In added value terms, the effect of the increased yields and reduced costs of production on farm income in 2008 was equivalent to an annual increase in production of 6.3% (165,400 tons).

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 17 Farm-level income impact of using GM IR cotton in the USA 1996–2008

Year	Cost savings (net after cost of technology ($/ha)	Net increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1996	4.98	115.32	94.69	1.19
1997	4.98	103.47	87.28	1.30
1998	4.98	88.54	80.62	1.47
1999	4.98	65.47	127.29	2.89
2000	4.98	74.11	162.88	3.10
2001	4.98	53.04	125.22	3.37
2002	4.98	69.47	141.86	3.11
2003	5.78	120.49	239.98	4.27
2004	5.78	107.47	261.23	4.82
2005	24.48	117.81	332.41	5.97
2006	−5.77	86.61	305.17	4.86
2007	−2.71	114.50	296.00	5.49
2008	−2.71	98.22	189.50	5.89

Sources and notes:

1. Impact data based on Gianessi and Carpenter [28], Sankala and Blumenthal [6, 7], Johnson and Strom [8], Marra et al. [3], and Mullins and Hudson [40]
2. Yield impact +9% 1996–2002 Bollgard I and +11% 2003 onward Bollgard II
3. Cost of technology: 1996–2002 Bollgard I $58.27/ha, 2003–2004 Bollgard II $68.32/ha, $49.62/ha 2005, $46.95/ha 2006, $25.7/ha 2007 and 2008
4. Insecticide cost savings $63.26/ha 1996–2002, $74.10/ha 2003–2005, $41.18/ha 2006, $28.4/ha 2007 and 2008

China

China first planted GM IR cotton in 1997, since when the area planted to GM IR varieties has increased to 64% of the total 5.95 million hectares crop in 2008.

As in the USA, a major farm income impact has been via higher yields of 8–10% on the crops using the technology, although there have also been significant cost savings on insecticides used and the labor previously used to undertake spraying. Overall, annual average costs have fallen by about $145–$200/ha and annual average profitability improved by $123–$472/ha. In 2008, the net national gain to farm income was $859 million (Table 18). Cumulatively, since 1997, the farm income benefit has been $7.6 billion. In added value terms, the effect of the increased yields and reduced costs of production on farm income in 2008 was equivalent to an annual increase in production of 17.1% (1.38 million tons).

Australia

Australia planted 83% of its 2008 cotton crop (total crop of 146,000 ha) to varieties containing GM IR traits (Australia first planted commercial GM IR cotton in 1996).

Unlike the other main countries using GM IR cotton, Australian growers have rarely derived yield gains from using the technology (reflecting the effective use of insecticides for pest control prior to the availability of GM IR cultivars), with the primary farm income benefit being derived from lower costs of

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 18 Farm-level income impact of using GM IR cotton in China 1997–2008

Year	Cost savings (net after cost of technology ($/ha)	Net increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1997	194	333	11.33	0.13
1998	194	310	80.97	1.15
1999	200	278	181.67	4.62
2000	−14	123	150.18	2.61
2001	378	472	1,026.26	20.55
2002	194	327	687.27	11.19
2003	194	328	917.00	12.15
2004	194	299	1,105.26	16.89
2005	145	256	845.58	13.57
2006	146	226	792.28	16.86
2007	152	248	942.7	14.46
2008	148	224	858.6	17.14

Sources and notes:

1. Impact data based on Pray et al. [41, 42], which covered the years 1999–2001. Other years based on average of the 3 years, except 2005 onward based on Shachuan (2006) – personal communication
2. Negative cost savings in 2000 reflect a year of high pest pressure (of pests not the target of GM IR technology), which resulted in above average use of insecticides on GM IR using farms
3. Yield impact +8% 1997–1999 and +10% 2000 onward
4. Negative value for the net cost savings in 2000 = a net increase in costs (i.e., the extra cost of the technology was greater than the savings on insecticide expenditure – a year of lower than average bollworm problems
5. All values for prices and costs denominated in Chinese Yuan have been converted to US dollars at the annual average exchange rate in each year

production (Table 19). More specifically, the following observations were made:

- In the first 2 years of adoption of the technology (Ingard, single gene Bt cotton), small net income losses were derived, mainly because of the relatively high price charged for the seed. Since this price was lowered in 1998, the net income impact has been positive, with cost saving of between $54/ha and $90/ha, mostly derived from lower insecticide costs (including application) more than offsetting the cost of the technology.
- For the last few years of use, Bollgard II cotton (containing 2 Bt genes) has been available offering effective control of a broader range of cotton pests. Despite the higher costs of this technology, users have continued to make significant net cost savings of $186–$212/ha.
- At the national level in 2008, the net farm income gains were $24.2 million and cumulatively since 1996 the gains have been $214.9 million.
- In added value terms, the effect of the reduced costs of production on farm income in 2008 was equivalent to an annual increase in production of 37% (105,000 tons).

Argentina

GM IR cotton has been planted in Argentina since 1998. In 2008, it accounted for 73% of total cotton plantings.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 19 Farm-level income impact of using GM IR cotton in Australia 1996–2008

Year	Cost of technology ($/ha)	Net increase in gross margins/cost saving after cost of technology ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1996	−191.7	−41.0	−1.63	−0.59
1997	−191.7	−35.0	−2.04	−0.88
1998	−97.4	91.0	9.06	0.43
1999	−83.9	88.1	11.80	4.91
2000	−89.9	64.9	10.71	4.38
2001	−80.9	57.9	7.87	5.74
2002	−90.7	54.3	3.91	3.43
2003	−119.3	256.1	16.3	11.49
2004	−179.5	185.8	45.7	21.33
2005	−229.2	193.4	47.9	23.75
2006	−225.9	190.7	22.49	26.01
2007	−251.33	212.1	11.73	40.90
2008	−264.26	199.86	24.23	37.40

Sources and notes:

1. Impact data based on Doyle [43], Taylor [44], CSIRO [45] for bollgard II since 2004
2. All values for prices and costs denominated in Australian dollars have been converted to US dollars at the annual average exchange rate in each year

The main impact in Argentina has been yield gains of 30% (which has resulted in a net increase in total cotton production (2008) of 22%). This has more than offset the cost of using the technology. In terms of gross margin, cotton farmers have gained annually between $25/ha and $249/ha during the period 1998–2007. At the national level, the annual farm income gains in the last 5 years have been in the range of $2–$27 million (Fig. 7). Cumulatively since 1998, the farm income gain from use of the technology has been $95.4 million. In added value terms, the effect of the yield increases (partially offset by higher costs of production) on farm income in 2008 was equivalent to an annual increase in production of 14.6%.

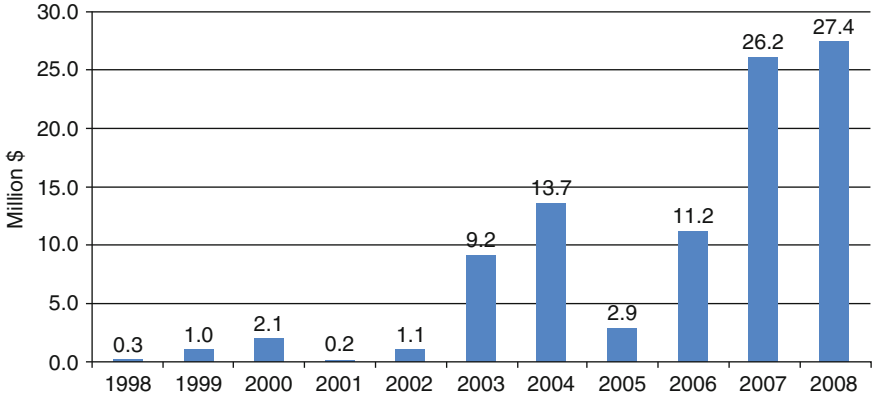
Mexico

GM IR cotton has been planted commercially in Mexico since 1996. In 2008, GM IR cotton was planted on 70,000 ha (56% of total cotton plantings).

The main farm income impact of using the technology has been yield improvements of between 6% and 9% over the last 6 years. In addition, there have been important savings in the cost of production (lower insecticide costs). Overall, the annual net increase in farm profitability has been within the range of $104/ha and $354/ha between 1996 and 2008 (Table 20). At the national level, the farm income benefit in 2008 was $10.5 million and the impact on total cotton production was an increase of 5.2%. Cumulatively since 1996, the farm income benefit has been $76.4 million. In added value terms, the combined effect of the yield increases and lower cost of production on farm income in 2008 was equivalent to an annual increase in production of 5.4%.

South Africa

In 2008, GM IR cotton (first planted commercially in 1998) was planted on 7,750 ha in South Africa (84% of the total crop).



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 7
National farm income impact of using GM IR cotton in Argentina 1998–2008
Sources and notes:
1. Impact data (Sources: Qaim and De Janvry [46, 47]), Elena [48] and for 2005 and 2006 Monsanto LAP, although cost of technology in 2005 from Monsanto Argentina. Area data: source ArgenBio
2. Yield impact +30%, cost of technology $86/ha ($40/ha 2005), cost savings (reduced insecticide use) $17.47/ha
3. All values for prices and costs denominated in Argentine Pesos have been converted to US dollars at the annual average exchange rate in each year

The main impact on farm incomes has been significantly higher yields (an annual average increase of about 24%). In terms of cost of production, the additional cost of the technology (between $17/ha and $24/ha for Bollgard I and $40–$50/ha for Bollgard II (2006 onward) has been greater than the insecticide cost and labor (for water collection and spraying) savings ($12–$23/ha), resulting in an increase in overall cost of production of $2–$32/ha. Combining the positive yield effect and the increase in cost of production, the net effect on profitability has been an annual increase of between $27/ha and $232/ha.

At the national level, farm incomes, over the last 5 years have annually increased by between $1.2 million and $1.7 million (Fig. 8). Cumulatively since 1998, the farm income benefit has been $21 million. The impact on total cotton production was an increase of 20.1% in 2008. In added value terms, the combined effect of the yield increases and lower costs of production on farm income in 2008 was equivalent to an annual increase in production of 14.5% (based on 2008 production levels).

India

GM IR cotton has been planted commercially in India since 2002. In 2008, 6.97 million hectares were planted to GM IR cotton, which is equal to 77% of total plantings.

The main impact of using GM IR cotton has been major increases in yield [54] found average yield increases of 45% in 2002 and 63% in 2003 (average over the 2 years of 54%) relative to conventionally produced cotton. More recent survey data from Monsanto [16] confirm this high-yield impact (+58% reported in 2004) as do data from IMRB [55], which found an average yield increase of 64% in 2005, and IMRB [56], which found a yield impact of +50% in 2006. With respect to cost of production, the average cost of the technology (seed premium: $49–$54/ha) up to 2006 was greater than the average insecticide cost savings of $31–$58/ha resulting in a net increase in costs of production. Following the reduction in the seed premium in 2006 to about $20/ha, farmers have, on average made a net cost saving of about $25/ha. Coupled with the yield gains, important net gains to levels of profitability have been achieved of between $82/ha and $356/ha. At the national level, the farm income gain in 2008 was $1.79 billion and cumulatively since 2002 the farm income gains have been $5.14 billion (Table 21).

The impact on total cotton production was an increase of 31% in 2008 and in added value terms, the combined effect of the yield increases and higher costs of production on farm income in 2008 was equivalent

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 20 Farm-level income impact of using GM IR cotton in Mexico 1996–2008

Year	Cost savings (net after cost of technology ($/ha)	Net increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
1996	58.1	354.5	0.32	0.1
1997	56.1	103.4	1.72	0.5
1998	38.4	316.4	11.27	2.71
1999	46.5	316.8	5.27	2.84
2000	47.0	262.4	6.85	5.76
2001	47.6	120.6	3.04	3.74
2002	46.1	120.8	1.84	3.81
2003	41.0	127.7	3.33	3.67
2004	39.3	130.4	6.24	4.51
2005	40.8	132.3	10.4	7.64
2006	20.4	124.4	6.44	4.06
2007	20.5	139.7	8.38	4.74
2008	19.9	150.4	10.52	5.44

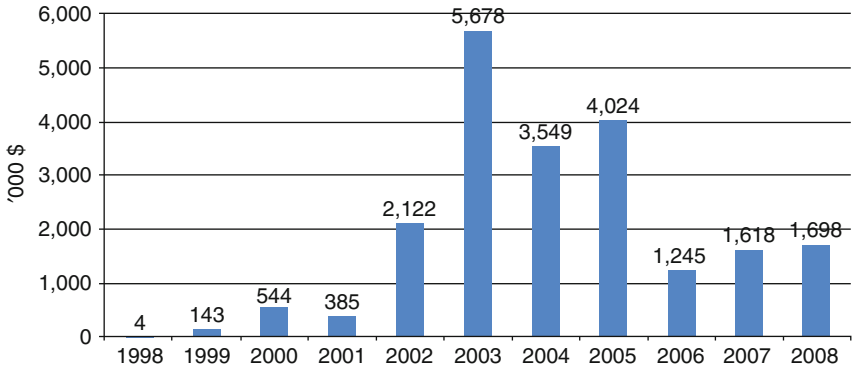
Sources and notes:

1. Impact data based on Traxler et al. [49] covering the years 1997 and 1998. Yield changes data in other years based on official reports submitted to the Mexican Ministry of Agriculture by Monsanto Comercial (Mexico). Also, Martinez-Carillo and Diaz-Lopez [50]
2. Yield impacts: 1996 +37%, 1997 +3%, 1998 +20%, 1999 +27%, 2000 +17%, 2001 +9%, 2002 +7%, 2003 +6%, 2004 +7.6%, 2005 onward +9.25%
3. All values for prices and costs denominated in Mexican Pesos have been converted to US dollars at the annual average exchange rate in each year

to an annual increase in production of 24% (based on the 2008 production level that is inclusive of the GM IR related yield gains).

Brazil

GM IR cotton was planted commercially in Brazil for the first time in 2006, and in 2008 was planted on 178,000 ha (20% of the total crop). This represents a fall in the share of total plantings relative to 2007, when GM IR traits were planted on 32% of the crop. This decline in plantings largely reflects the relative performance of the seed containing the GM IR traits compared to the leading conventional varieties, in which the GM IR trait has not been available. In 2006, on the basis of industry estimates of impact of GM IR cotton relative to similar varieties, an average yield gains of +6% and a net cost saving (reduced expenditure on insecticides after deduction of the premium paid for using the technology) of about +$25/ha were realized. In 2007 and 2008, however, analysis by Galveo [12] and Monsanto Brazil [57] suggests that the yield performance of the varieties containing GM IR traits has been lower (by −3.6% and −2.7% respectively for 2007 and 2008). As a result, the net farm income of using the technology was (after taking into consideration insecticide cost savings and the seed premium), on average, −$34.5/ha in 2007 and a small net gain of about $2/ha in 2008. At a national level in 2008, GM IR cotton technology delivered a net gain of about $0.35 million (a net loss of $12.3 million in 2007). Cumulatively, the total farm income impact has been positive at about $5 million.



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 8
National farm income impact of using GM IR cotton in South Africa 1998–2008
Sources and notes:
1. Impact data based on Ismael et al. [51], Kirsten et al. [52], Morse et al. [53]
2. Yield impact +24%, cost of technology $14–$24/ha for Bollgard I and about $50/ha for Bollgard II, cost savings (reduced insecticide use) $12–$23/ha
3. All values for prices and costs denominated in South African Rand have been converted to US dollars at the annual average exchange rate in each year
4. The decline in the total farm income benefit post 2003 relative to earlier years reflects the decline in total cotton plantings. This was caused by relatively low farm-level prices for cotton in 2004 and 2005 (reflecting a combination of relatively low world prices and a strong South African currency). In more recent years, cotton has become less competitive relative to alternatives such as corn because of higher world grain prices

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 21 Farm-level income impact of using GM IR cotton in India 2002–2008

Year	Cost savings (net after cost of technology ($/ha)	Net increase in gross margins ($/ha)	Increase in farm income at a national level ($ millions)	Increase in national farm income as % of farm-level value of national production
2002	−12.42	82.66	3.69	0.26
2003	−16.2	209.85	20.98	0.47
2004	−13.56	193.36	96.68	1.86
2005	−22.25	255.96	332.74	5.26
2006	3.52	221.02	839.89	14.04
2007	26.41	356.85	2,093.97	22.84
2008	24.28	256.73	1,790.16	24.27

Sources and notes:
1. Impact data based on Bennett et al. [54] and IMRB [55, 56]. As 2008 was reported to be a year of below average pest pressure, the average yield gain used was reduced to +40%
2. All values for prices and costs denominated in Indian Rupees have been converted to US dollars at the annual average exchange rate in each year

Other Countries

- *Colombia*: GM IR cotton has been grown commercially in Colombia since 2002 (20,000 ha planted in 2008 out of a total cotton crop of 40,000 ha). Drawing on recent analysis of impact by Zambrano et al. [58], this shows the main impact has been through a significant improvement in yields of +32%. On the cost impact side, this analysis shows that farmers using GM IR cotton tend to have substantially higher expenditures on pest control than their conventional counterparts, which when taking into consideration the approximate $70/ha cost of the technology results in a net addition to costs of between $200/ha and $280/ha each year (relative to typical expenditures by conventional cotton growers). Nevertheless, after taking into consideration the positive yield effects, the net impact on profitability has been positive. In 2008, the average improvement in profitability was about $33/ha and the total net gain from using the technology was $0.91 million. Cumulatively, since 2002, the net farm income gain has been $13.9 million.
- *Burkino Faso*: GM IR cotton was grown commercially first in 2008. Based on analysis of precommercial trials by Vitale et al. [59, 60], the main impact of the technology is improved yields (by +20%) and savings in insecticide expenditure of about $62/ha. Based on a cost of technology of about $42/ha, the net cost savings are about $20/ha, and inclusive of the yield gains, the estimated net income gain in 2008 was $124/ha. The total aggregate farm income gain in 2008 was therefore $1 million.

Summary of Global Impact

In global terms, the farm-level impact of using GM IR cotton was $2.9 billion in 2008. Cumulatively, since 1996, the farm income benefit has been (in nominal terms) $15.61 billion. Within this, 65% of the farm income gain has derived from yield gains (less pest damage) and the balance (35%) from reduced expenditure on crop protection (spraying of insecticides).

In terms of the total value of cotton production from the countries growing GM IR in 2008, the additional farm income generated by the technology is equal to a value-added equivalent of 19.3% (based on the 2008 production level inclusive of the GM IR related yield gains). Relative to the value of global cotton production in 2008, the farm income benefit added the equivalent of 11.1%.

Other Biotech Crops

Maize/Corn Rootworm Resistance

GM rootworm-resistant (CRW) corn has been planted commercially in the USA since 2003. In 2008, there were 13.7 million hectares of CRW corn (43% of the total US crop).

The main farm income impact (Impact data based on Sankala and Blumenthal [6, 7], Johnson and Strom [8], Rice [61]), and Alston et al. [62]) has been higher yields of about 5% relative to conventional corn. The impact on average costs of production has been +$2/ha to −$10/ha (based on an average cost of the technology of $35–$42/ha and an insecticide cost saving of $32–$37/ha). As a result, the net impact on farm profitability has been +$28/ha to +$79/ha.

At the national level, farm incomes increased by $4.6 million in 2003, rising to $1.1 billion in 2008. Cumulatively since 2003, the total farm income gain from the use of CRW technology in the USA corn crop has been $2 billion.

CRW cultivars were also planted commercially for the first time in 2004 in Canada. In 2008, the area planted to CRW-resistant varieties was 119,380 ha. Based on US costs, insecticide cost savings and yield impacts, this has resulted in additional income at the national level of $8.65 million in 2008 (cumulative total since 2004 of $13 million).

At the global level, the extra farm income derived from biotech CRW maize use since 2003 has been just over $2 billion. In 2008, the additional farm income generated from use of the technology was equal to 0.9% of the value of the global maize crop.

Virus-Resistant Papaya

Ring spot-resistant papaya has been commercially grown in the USA (State of Hawaii) since 1999, and in 2008 (85% of the state's papaya crop was GM virus resistant (700 ha).

The main farm income impact of this biotech crop has been to significantly increase yields relative to conventional varieties. Compared to the average yield in the last year before the first biotech cultivation (1998), the annual average yield increase of biotech papaya relative to conventional crops has been within a range of +15% to +77% (29% in 2008). At a state level, this is equivalent to a 25% increase in total papaya production in 2008.

In terms of profitability (Impact data based on Sankala and Blumenthal [6, 7] and Johnson and Strom [8]), the net annual impact has been an improvement of between $3,000/ha and $29,000/ha, and in 2008 this amounted to a net farm income gain of $5,790/ha and an aggregate benefit across the state of $4 million. Cumulatively, the farm income benefit since 1999 has been $53.4 million.

Virus-resistant papaya is also reported to have been grown in China in 2008, on 4,500 ha. No impact data on this technology has been identified.

Virus-Resistant Squash

Biotech virus-resistant squash has also been grown in some states of the USA since 2004 and is estimated to have been planted on 2,900 ha in 2008 (17% of the total crop in the USA – mostly found in Georgia and Florida).

Based on analysis from Johnson and Strom [8], the primary farm income impact of using biotech virus-resistant squash has been derived from higher yields, which in 2008, added a net gain to users of $26 million. Cumulatively, the farm income benefit since 2004 has been $107 million.

Insect-Resistant Potatoes

GM insect-resistant potatoes were also grown commercially in the USA between 1996 and 2000 (planted on 4% of the total US potato crop in 1999 (30,000 ha). This technology was withdrawn in 2001 when the technology provider (Monsanto) withdrew from the market to concentrate on GM trait development in maize, soybeans, cotton, and canola. This commercial decision was also probably influenced by the decision of some leading potato processors and fast-food outlets to stop using GM potatoes because of perceived concerns about this issue from some of their consumers, even though the GM potato provided the producer and the processor with a lower cost, higher yielding, and more consistent product. It also delivered significant reductions in insecticide use Carpenter and Gianessi (2002).

Indirect (Nonpecuniary) Farm-Level Economic Impacts

Apart from the tangible and quantifiable impacts on farm profitability presented above, there are other important, more intangible (difficult to quantify) impacts of an economic nature.

Many of the studies of the impact of biotech crops have identified the following reasons as being important influences for adoption of the technology:

Herbicide-Tolerant Crops

- Increased management flexibility and convenience that comes from a combination of the ease of use associated with broad-spectrum, post-emergent herbicides like glyphosate and the increased/longer time window for spraying. This not only frees up management time for other farming activities but also allows additional scope for undertaking off-farm, income-earning activities.
- In a conventional crop, post-emergent weed control relies on herbicide applications before the weeds and crop are well established. As a result, the crop may suffer "knock-back" to its growth from the effects of the herbicide. In the GM HT crop, this problem is avoided because the crop is both tolerant to the herbicide and spraying can occur at a later stage when the crop is better able to withstand any possible "knock-back" effects.
- Facilitates the adoption of conservation or no tillage systems. This provides for additional cost savings such as reduced labor and fuel costs associated with plowing, additional moisture retention, and reductions in levels of soil erosion.
- Improved weed control has contributed to reduced harvesting costs – cleaner crops have resulted in reduced times for harvesting. It has also improved harvest quality and led to higher levels of quality price bonuses in some regions and years (e.g., HT soybeans and HT canola in the early years of adoption respectively in Romania and Canada).

- Elimination of potential damage caused by soil-incorporated residual herbicides in follow-on crops and less need to apply herbicides in a follow-on crop because of the improved levels of weed control.
- A contribution to the general improvement in human safety (as manifest in greater peace of mind about own and worker safety) from reduced exposure to herbicides and a switch to more environmentally benign products.

Insect-Resistant Crops

- Production risk management/insurance purposes – the technology takes away much of the worry of significant pest damage occurring and is, therefore, highly valued. Piloted in 2008 and more widely operational from 2009, US farmers using stacked corn traits (containing insect resistance and herbicide-tolerant traits) are being offered discounts on crop insurance premiums equal to $7.41/ha.
- A "convenience" benefit derived from having to devote less time to crop walking and/or applying insecticides.
- Savings in energy use – mainly associated with less use of aerial spraying and less tillage.
- Savings in machinery use (for spraying and possibly reduced harvesting times).
- Higher quality of crop. There is a growing body of research evidence relating to the superior quality of GM IR corn relative to conventional and organic corn from the perspective of having lower levels of mycotoxins. Evidence from Europe (as summarized in Brookes [35] has shown a consistent pattern in which GM IR corn exhibits significantly reduced levels of mycotoxins compared to conventional and organic alternatives. In terms of revenue from sales of corn, however, no premia for delivering product with lower levels of mycotoxins have, to date, been reported although where the adoption of the technology has resulted in reduced frequency of crops failing to meet maximum permissible fumonisin levels in grain maize (e.g., in Spain), this delivers an important economic gain to farmers selling their grain to the food using sector. GM IR corn farmers in the Philippines have also obtained price premia of 10% [37] relative to conventional corn because of better quality, less damage to cobs and lower levels of impurities.
- Improved health and safety for farmers and farm workers (from reduced handling and use of pesticides, especially in developing countries where many apply pesticides with little or no use of protective clothing and equipment).
- Shorter growing season (e.g., for some cotton growers in India), which allows some farmers to plant a second crop (notably maize) in the same season. Also some Indian cotton growers have reported knock on benefits for beekeepers as fewer bees are now lost to insecticide spraying [63].

Some of the economic impact studies have attempted to quantify some of these benefits (e.g., Qaim and Traxler [9] quantified some of these in Argentina (a $3.65/ha saving (−7.8%) in labor costs and a $6.82/ha (−28%) saving in machinery/fuel costs associated with the adoption of GM HT soybeans). Where identified, these cost savings have been included in the analysis presented above. Nevertheless, it is important to recognize that these largely intangible benefits are considered by many farmers as a primary reason for adoption of GM technology, and in some cases farmers have been willing to adopt for these reasons alone, even when the measurable impacts on yield and direct costs of production suggest marginal or no direct economic gain.

Since the early 2000s, a number of farmer-survey based studies in the USA have also attempted to better quantify these nonpecuniary benefits. These studies have usually employed contingent valuation techniques to obtain farmers valuations of nonpecuniary benefits. A summary of these findings is shown in (Table 22).

Aggregating the Impact to US Crops 1996–2008

The approach used to estimate the nonpecuniary benefits derived by US farmers from biotech crops over the period 1996–2008 has been to draw on the values identified by Marra and Piggot ([1, 2]: Table 22) and to apply these to the biotech crop planted areas during this 13-year period. Figure 9 summarizes the values for nonpecuniary benefits derived from biotech crops in the USA (1996–2008) and shows an estimated (nominal value) benefit of $855 million in

2008 and a cumulative total benefit (1996–2008) of $5.99 billion. Relative to the value of direct farm income benefits presented above, the nonpecuniary benefits were equal to 21% of the total direct income benefits in 2008 and 25.6% of the total cumulative (1996–2008) direct farm income. This highlights the important contribution this category of benefit has had on biotech trait adoption levels in the USA, especially where the direct farm income benefits have been identified to be relatively small (e.g., HT cotton).

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 22 Values of nonpecuniary benefits associated with biotech crops in the USA

Survey	Median value ($/ha)
2002 IR (to rootworm) corn growers survey	7.41
2002 soybean (HT) farmers survey	12.35
2003 HT cropping survey (corn, cotton, and soybeans) – North Carolina	24.71
2006 HT (flex) cotton survey	12.35 (relative to first generation HT cotton)

Source: Marra and Piggot 2006 and 2007 [1, 2]

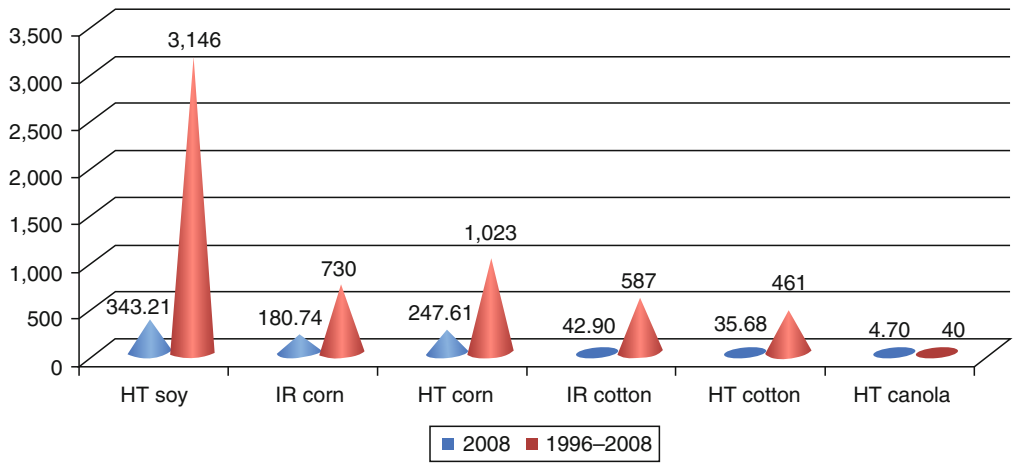
Estimating the Impact in Other Countries

It is evident from the literature review that farmers in other countries, who use GM technology, also value the technology for a variety of nonpecuniary/intangible reasons. The most appropriate methodology for identifying these nonpecuniary benefit valuations in other countries would be to repeat the type of US farmer surveys in other countries. Unfortunately, the authors are not aware of any such studies having been undertaken to date.

Production Effects of the Technology

Based on the yield assumptions used in the direct farm income benefit calculations presented above and taking account of the second soybean crop facilitation in South America, biotech crops have added important volumes to global production of corn, cotton, canola, and soybeans since 1996 (Table 23).

The biotech IR traits, used in the corn and cotton sectors, have accounted for 99% of the additional corn production and almost all of the additional cotton production. Positive yield impacts from the use of this technology have occurred in all user countries (except GM IR cotton in Australia: this reflects the levels of *Heliothis* pest control previously obtained with intensive insecticide use. The main benefit and reason for adoption of this technology in Australia has arisen from significant cost savings (on insecticides) and the associated environmental gains from reduced insecticide use)



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 9
Nonpecuniary benefits derived by US farmers 1996–2008 by trait ($ million)

when compared to average yields derived from crops using conventional technology (such as application of insecticides and seed treatments). Since 1996, the average yield impact across the total area planted to these traits over the 12-year period has been +7.1% for corn traits and +14.8% for cotton traits (Fig. 10).

Although the primary impact of biotech HT technology has been to provide more cost-effective (less expensive) and easier weed control versus improving yields from better weed control (relative to weed control obtained from conventional technology), improved weed control has, nevertheless occurred, delivering higher yields in some countries. Specifically, HT soybeans in Romania improved the average yield by over 30% in early adoption years and biotech HT corn in Argentina and the Philippines delivered yield improvements of +9% and +15% respectively.

Biotech HT soybeans have also facilitated the adoption of no tillage production systems, shortening the production cycle. This advantage enables many farmers in South America to plant a crop of soybeans immediately after a wheat crop in the same growing season. This second crop, additional to traditional soybean production, has added 73.5 million tons to soybean production in Argentina and Paraguay between 1996 and 2008 (accounting for 99% of the total biotech-related additional soybean production).

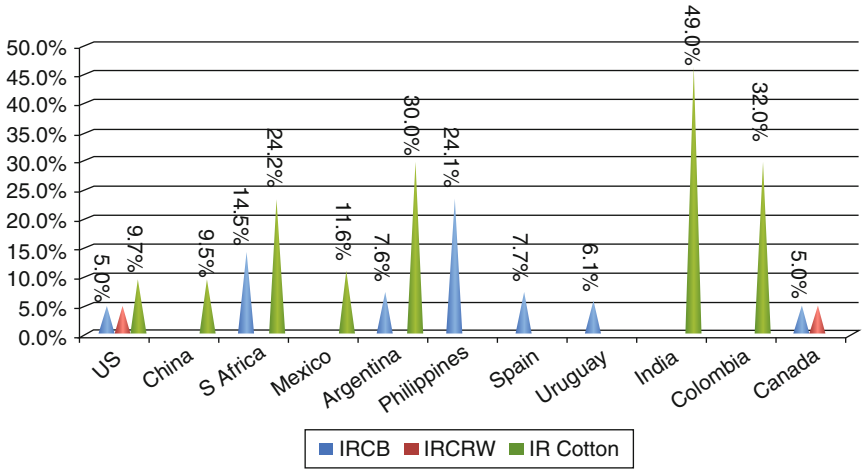
Using the same sensitivity analysis as applied to the farm income estimates presented in the executive summary to the production impacts (one scenario of consistent lower than average pest/weed pressure and one of consistent higher than average pest/weed pressure), Table 24 shows the range of production impacts.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 23 Additional crop production arising from positive yield effects of biotech crops

	1996–2008 additional production (million tons)	2008 additional production (million tons)
Soybeans	74.0	10.1
Corn	79.7	17.1
Cotton	8.6	1.8
Canola	4.8	0.6

Summary of Economic Effects of Transgenic/Biotech Crops

Overall, GM technology has had a significant positive impact on farm income derived from a combination of enhanced productivity and efficiency gains (Table 25). In 2008, the direct global farm income benefit from biotech crops was $9.37 billion. This is equivalent to having added 3.6% to the value of global production of



Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Figure 10
Average yield impact of biotech IR traits 1996–2008 by country and trait Notes: IRCB, resistant to corn-boring pests; IRCRW, resistant to corn rootworm

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 24 Additional crop production arising from positive yield effects of biotech crops 1996–2008 under different pest/weed pressure assumptions and impacts of the technology (million tons)

Crop	Consistent below average pest/weed pressure	Average pest/weed pressure (main study analysis)	Consistent above average pest/weed pressure
Soybeans	73.8	74.0	74.3
Corn	48.0	79.7	140.9
Cotton	6.2	8.6	11.8
Canola	3.3	4.8	5.2

Note: No significant change to soybean production under all three scenarios as 99% of production gain due to second cropping facilitation of the technology

the four main crops of soybeans, maize, canola, and cotton. Since 1996, farm incomes have increased by $52 billion.

The largest gains in farm income have arisen in the soybean sector, largely from cost savings. The $2.93 billion additional income generated by GM herbicide-tolerant (GM HT) soybeans in 2008 has been equivalent to adding 4.3% to the value of the crop in the biotech growing countries, or adding the equivalent of 4.1% to the $71 billion value of the global soybean crop in 2008. These economic benefits should, however be placed within the context of a significant increase in the level of soybean production in the main biotech adopting countries. Since 1996, the soybean area in the leading soybean producing countries of the USA, Brazil, and Argentina increased by 63%.

Substantial gains have also arisen in the cotton sector mainly from the adoption of GM insect-resistant (GM IR) cotton (through a combination of higher yields and lower costs). In 2008, cotton farm income levels in the biotech adopting countries increased by $2.9 billion and since 1996, the sector has benefited from an additional $15.6 billion. The 2008 income gains are equivalent to adding 19.3% to the value of the cotton crop in these countries, or 11.1% to the $26 billion value of total global cotton production. This is a substantial increase in value-added terms for two new cottonseed technologies.

Significant increases to farm incomes have also resulted in the maize and canola sectors. The combination of GM insect resistant (GM IR) and GM HT technology in maize has boosted farm incomes by $10.24 billion since 1996. In the canola sector (largely North American) an additional $1.83 billion has been generated.

Of the total cumulative farm income benefit, $31.2 billion (60%) has been due to yield gains (and second crop facilitation), with the balance arising from reductions in the cost of production. Within this yield gain component, 76% derives from the GM IR technology and the balance to GM HT crops.

Table 26 summarizes farm income impacts in key biotech adopting countries. This highlights the important farm income benefit arising from GM HT soybeans in South America (Argentina, Brazil, Paraguay, and Uruguay), GM IR cotton in China and India, and a range of GM cultivars in the USA. It also illustrates the growing level of farm income benefits obtained in South Africa, the Philippines, and Mexico.

In terms of the division of the economic benefits obtained by farmers in developing countries relative to farmers in developed countries, Table 27 shows that in 2008, 50.5% of the farm income benefits have been earned by developing country farmers. The vast majority of these income gains for developing country farmers have been from GM IR cotton and GM HT soybeans. Over the 13 years, 1996–2008, the cumulative farm income gain derived by developing country farmers was also 50% ($26.2 billion).

Examining the cost farmers pay for accessing GM technology, Table 28 shows that across the four main biotech crops, the total cost in 2008 was equal to 27% of the total technology gains (inclusive of farm income gains plus cost of the technology payable to the seed supply chain: the cost of the technology accrues to the seed supply chain including sellers of seed to farmers, seed multipliers, plant breeders, distributors, and the GM technology providers).

For farmers in developing countries the total cost was equal to 15% of total technology gains, while for farmers in developed countries the cost was 36% of the total technology gains. While circumstances vary between countries, the higher share of total technology gains accounted for by farm income gains in developing countries relative to the farm income share in developed countries reflects factors such as

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 25 Global farm income benefits from growing biotech crops 1996–2008: million US $

Trait	Increase in farm income 2008	Increase in farm income 1996–2008	Farm income benefit in 2008 as % of total value of production of these crops in biotech adopting countries	Farm income benefit in 2008 as % of total value of global production of crop
GM herbicide-tolerant soybeans	2,925.7	23,342.0	4.3	4.1
GM herbicide-tolerant maize	433.5	1,896.0	0.6	0.3
GM herbicide-tolerant cotton	14.6	855.8	0.1	0.06
GM herbicide-tolerant canola	391.8	1,829.2	6.9	1.5
GM insect-resistant maize	2,645.5	8,344.2	3.7	2.0
GM insect-resistant cotton	2,904.5	15,612.7	19.3	11.1
Others	51.5	162.1	Not applicable	Not applicable
Totals	9,367.1	52,042.0	5.71	3.65

Notes: All values are nominal. Others = Virus-resistant papaya and squash and herbicide-tolerant sugar beet. Totals for the value shares exclude "other crops" (i.e., relate to the four main crops of soybeans, maize, canola, and cotton). Farm income calculations are net farm income changes after inclusion of impacts on yield, crop quality, and key variable costs of production (e.g., payment of seed premia, impact on crop protection expenditure)

weaker provision and enforcement of intellectual property rights in developing countries and the higher average level of farm income gain on a per hectare basis obtained by farmers in developing countries relative to that obtained by farmers in developed countries.

Concluding Comments

Biotechnology has, to date delivered several specific agronomic traits that have overcome a number of production constraints for many farmers. This has resulted in improved productivity and profitability for the 13.3 million adopting farmers who have applied the technology to 115 million hectares in 2008.

During the last 13 years, this technology has made important positive socioeconomic and environmental contributions. These have arisen even though only a limited range of biotech agronomic traits have so far been commercialized, in a small range of crops.

The biotechnology has delivered economic and environmental gains through a combination of their inherent technical advances and the role of the

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 26 GM crop farm income benefits 1996–2008 selected countries: million US $

	GM HT soybeans	GM HT maize	GM HT cotton	GM HT canola	GM IR maize	GM IR cotton	Total
The USA	11,028	1,705.6	799	185.0	7,107	2,444.1	*23,268.7*
Argentina	8,764.1	113.8	34.2	N/a	269.8	95.4	*9,277.3*
Brazil	2,745.8	N/a	N/a	N/a	69.8	5.0	*2,820.6*
Paraguay	503.2	N/a	N/a	N/a	N/a	N/a	*503.2*
Canada	116.1	45.8	N/a	1,643.2	265.4	N/a	*2,070.5*
South Africa	4.1	3.8	2.2	N/a	475.8	21.0	*506.9*
China	N/a	N/a	N/a	N/a	N/a	7,599	*7,599*
India	N/a	N/a	N/a	N/a	N/a	5,142	*5,142*
Australia	N/a	N/a	8.3	0.9	N/a	214.9	*224.1*
Mexico	3.3	N/a	11.7	N/a	N/a	76.1	*91.1*
The Philippines	N/a	27.1	N/a	N/a	61.2	N/a	*88.3*
Romania	44.6	N/a	N/a	N/a	N/a	N/a	*44.9*
Uruguay	49.4	N/a	N/a	N/a	3.9	N/a	*53.3*
Spain	N/a	N/a	N/a	N/a	77.9	N/a	*77.9*
Other EU	N/a	N/a	N/a	N/a	11.1	N/a	*11.1*
Columbia	N/a	N/a	N/a	N/a	N/a	13.9	*13.9*
Bolivia	83.4	N/a	N/a	N/a	N/a	N/a	*83.4*

Notes: All values are nominal. Farm income calculations are net farm income changes after inclusion of impacts on yield, crop quality, and key variable costs of production (e.g., payment of seed premia, impact on crop protection expenditure). N/a = not applicable. US total figure excludes $182.3 million for other crops/traits

technology in the facilitation and evolution of more cost-effective and environmentally friendly farming practices. More specifically, it covers the following main issues:

- The gains from the GM IR traits have mostly been delivered directly from the technology (yield improvements, reduced production risk, and decreased the use of insecticides). Thus farmers (mostly in developing countries) have been able to both improve their productivity and economic returns while also practicing more environmentally friendly farming methods.
- The gains from GM HT traits have come from a combination of direct benefits (mostly cost reductions to the farmer) and the facilitation of changes in farming systems. Thus, GM HT technology (especially in soybeans) has played an important role in enabling farmers to capitalize on the availability of a low-cost, broad-spectrum herbicide (glyphosate) and in turn, facilitated the move away from conventional to low/no tillage production systems in both North and South America. This change in production system has made additional positive economic contributions to farmers (and the wider economy) and delivered important environmental benefits, notably reduced levels of GHG emissions (from reduced tractor fuel use and additional soil carbon sequestration).
- Both IR and HT traits have made important contributions to increasing world production levels of soybeans, corn, cotton, and canola.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 27 GM crop farm income benefits 2008: developing versus developed countries: million US $

	Developed	Developing
GM HT soybeans	1,232.1	1,693.6
GM IR maize	2,380.5	265.0
GM HT maize	357.4	76.1
GM IR cotton	213.8	2,690.8
GM HT cotton	5.5	9.1
GM HT canola	391.8	0
GM virus-resistant papaya and squash and GM HT sugar beet	51.5	0
Total	4,632.6	4,734.6

Developing countries = all countries in South America, Mexico, Honduras, Burkino Faso, India, China, the Philippines, and South Africa

The impact of GM HT traits has, however contributed to increased reliance on a limited range of herbicides and this has contributed to some limited development of weed resistance to these herbicides. Some degree of reduced effectiveness of glyphosate (and glufosinate) against certain weeds is to be expected and the extent to which this may develop further, will depend on farming practice and behavior relating to mixing, rotation, and sequencing of herbicides. Where resistance has occurred, this has resulted in low-dose rate applications of other herbicides in weed control programs (commonly used in conventional production systems) occurring and hence, has marginally reduced the level of net environmental and economic gains derived from the current use of the biotechnology. Nevertheless, to date, the overall environmental and economic gains arising from the use of biotech crops have been substantial.

Global Economic Impact of Transgenic/Biotech Crops (1996–2008). Table 28 Cost of accessing GM technology (million $) relative to the total farm income benefits 2008

	Cost of technology: all farmers	Farm income gain: all farmers	Total benefit of technology to farmers and seed supply chain	Cost of technology: developing countries	Farm income gain: developing countries	Total benefit of technology to farmers and seed supply chain: developing countries
GM HT soybeans	1,058.2	2,925.7	3,983.9	334.4	1,693.6	2,028.0
GM IR maize	1,045.9	2,645.5	3,691.4	99.7	265.0	364.7
GM HT maize	547.8	433.5	981.3	32.5	76.1	108.6
GM IR cotton	434.6	2,904.5	3,339.1	353.0	2,690.8	3,043.8
GM HT cotton	167.1	14.6	181.7	10.4	9.1	19.5
GM HT canola	109.0	391.8	500.86	N/a	N/a	N/a
Others	41.5	51.5	93.0	N/a	N/a	N/a
Total	3,404.1	9,367.1	12,771.26	830.0	4,734.6	5,564.6

N/a, not applicable. Cost of accessing technology based on the seed premia paid by farmers for using GM technology relative to its conventional equivalents

Appendix 1: Argentine Second Crop Soybeans

Year	Second crop area (million hectares)	Increase in income linked to GM HT system (million $)	Additional production (million tons)
1996	0.45	Negligible	Negligible
1997	0.65	25.4	0.3
1998	0.8	43.8	0.9
1999	1.4	116.6	2.3
2000	1.6	144.2	2.7
2001	2.4	272.8	5.7
2002	2.7	372.6	6.9
2003	2.8	416.1	7.7
2004	3.0	678.1	6.9
2005	2.3	526.7	6.3
2006	3.2	698.9	11.2
2007	4.9	1,133.6	9.88
2008	3.4	764.6	9.62

Additional gross margin based on data from Grupo CEO

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GM Crop Risk Debate, Science and Socioeconomics

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Article Outline

Introduction

The General Strategic Situation of the Debate About Green Biotechnology Today

The aim of this text is to set the framework for a better communication about science and regulation, and production of GM crops. GM stands for Genetic Modification, basically an unfortunate denomination, because actually *all* crops are genetically modified, but it is a worldwide accepted term for genetically engineered crops, including transgenes, auto- and allotransgenes, cis- and infra-genes, and synthetic genes, for details see Beardmore [1]. By including gene stacking of various kinds, the situation is getting even more complex [2]. With the introduction of in Vivo Mutation (with Zink-Finger Technology and the latest transformation method transcription activator-like family of type III effectors [TALEs]) the situation will change even more, the age of a high precision and targeted change of genomes has only begun and will develop rapidly, see section Innovation in Agriculture on All Levels Will Speed Up and Makes it a Necessity to Rethink Regulation Basically and Radically, Most often in the Direction of Lowering the Regulatory Hurdles with details. The term LMOs (Living Modified Organisms), which is generally used in the United Nations Biosafety Protocol (Cartagena Protocol) is nothing but a "Living Proof" that the scientific basis of the Protocol remains questionable, since firstly the term is creating misunderstandings and secondly it is based on an erroneous assumption that GM crops are basically different from conventional crops, as is discussed with detail in the sections Molecular Processes Similar in Natural Mutation and Transgenesis and Dissent over Differences Between GM- and Non-GM Crops Causes Transatlantic Regulatory Divide. More detailed clarification about the terminology of GMOs is given in a text block of the published Statement of the Pontifical Academy of Sciences: [3].

- There are many different terms used to describe the processes involved in plant breeding. All living organisms are made up of cells in which are contained their genes, which give them their distinctive characteristics. The complete set of genes (the genotype) is encoded in DNA and is referred to as the genome; it is the hereditary information that is passed from parent to offspring. All plant breeding, and indeed all evolution, involves genetic change or modification followed by selection for beneficial characteristics from among the offspring. Most alterations to a plant's phenotype or observable traits (such as its physical structure, development, biochemical and nutritional properties) result from changes to its genotype. Plant breeding traditionally used the random reshuffling of genes among closely-related and sexually compatible species, often with unpredictable consequences and always with the details of the genetic changes unexplored. In the mid-twentieth century this was supplemented by mutagenesis breeding, the equally random treatment of seeds or whole plants with mutagenic chemicals or high-energy radiation in the hope of generating phenotypic improvements; this, too, gave rise to unpredictable and unexplored genetic consequences from which the plant breeder selected the beneficial traits. Most recently, techniques have been developed allowing the transfer of specific, identified and well characterized genes, or small blocks of genes that confer particular traits, accompanied by a precise analysis of the genetic and phenotypic outcomes: this last category is called 'transgenesis' (because genes are transferred from a donor to a recipient) or 'genetic engineering' (abbreviated to GE in this report) but, in truth, this term applies to all breeding procedures.

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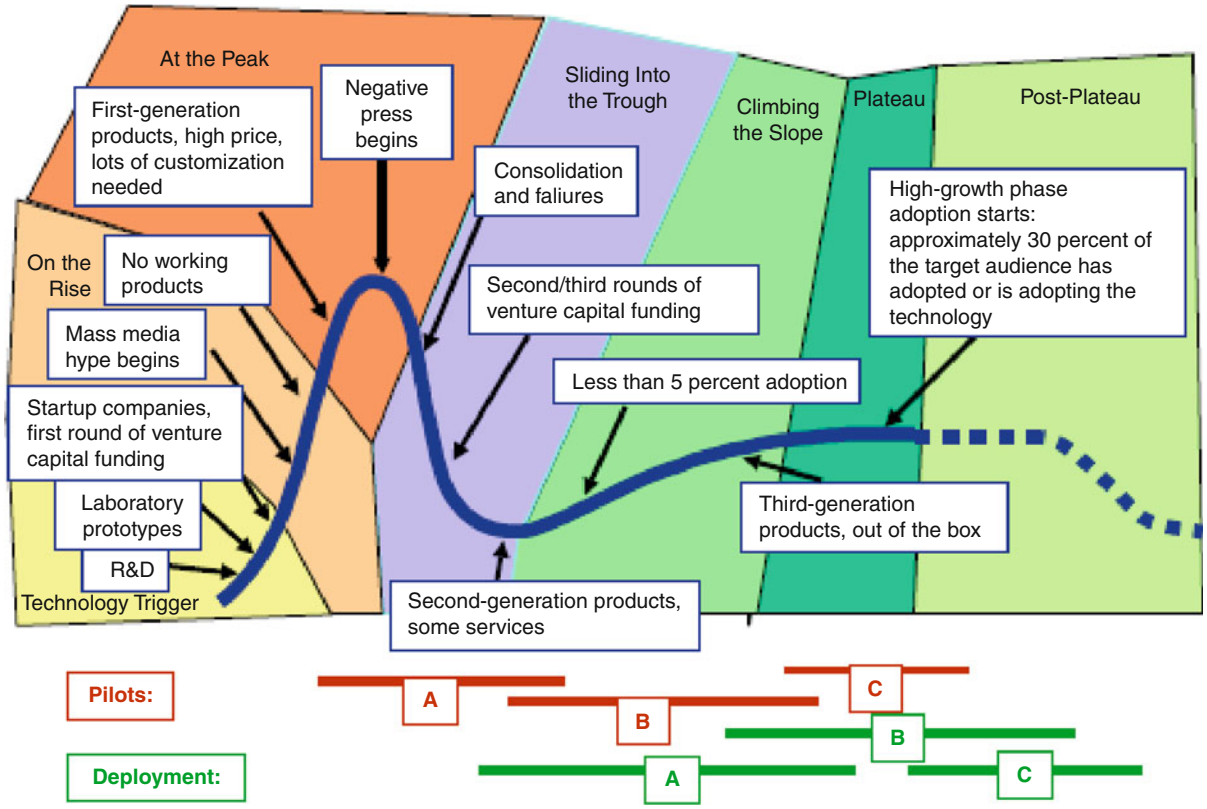


GM Crop Risk Debate, Science and Socioeconomics. Figure 1
Gartner Hype cycle, extended view from [6] after Fig. 3. Technologically aggressive ("Type A") enterprises are relatively comfortable adopting the technology, and moderately aggressive ("Type B") enterprises start to investigate and pilot the technology. Conservative ("Type C") enterprises remain wary (From [6])

The strategic situation in the debate on GM crops is difficult, but not desperate, particularly in Europe – this is an evaluation shared by lots of experts of the debate about agricultural biotechnology; in Europe, it is negatively affecting research and researchers [4]. We have reached in Europe the peak of anxiety related to GM-crops since the introduction of the new technologies, and some opponents to transgenic crops have taken advantage of this situation. They have organized themselves in a veritable protest industry, see section The Dispute Between Scientists and Opponents Today. Nevertheless, the next years should lead to reassurance and scientific consolidation on biotechnology views. We encounter the same repeating dynamics as described for previous technology introductions [5]. The Gartner Hype Cycle [6] adds another dimension to technology life cycle models: it characterizes the typical progression of an emerging technology from user and media overenthusiasm through a period of disillusionment to an eventual understanding of the technology's relevance and role in a market or domain (Fig. 1).

In the details of the cycle [6], amended by the author – specified for the technology push in transgenic cropdevelopment – it should be noted that there are differences between the development of the technologies in the mind of Linden and Fenn and agricultural technologies, where life sciences, combined with regional and cultural diversity, results in a much more diversified picture, often not following the below described phases.

- **2.1 Technology Trigger.** The Technology Trigger is a technological breakthrough, public demonstration, press release or other event that generates significant publicity and industry interest in an emerging technology. Typically no usable products exist, only research and laboratory prototypes (from the first transgenic plants in the 80ties [7]. Venture capitalists may provide some early funding just after the Trigger, if they expect the technology to be a fast runner.

- **2.2 On the Rise.** On the rise to the Peak of Inflated Expectations, media articles explain the technology and discuss its potential impact on business and society. First-generation products emerge like the Flavr-Savr-Tomato [8], but they usually are highly specialized products or extremely difficult to use or with other hitches in the introductory phase. Products are high margin because vendors are still trying to recover R&D costs, and the technology is expensive compared to its cost of production. For example, in 2002, Bluetooth products such as headsets cost $200, while the final silicon cost of Bluetooth chips likely will be approximately $5. This is a good stage for venture capitalists to enter the market, before evaluations are at their apex. During this phase, some particularly aggressive enterprises may start to pilot the technology, particularly if it contributes to critical business issues. These enterprises work closely with the vendors to create customized solutions for their requirements

- **2.3 At the Peak of Inflated Expectations.** As the Peak crests, the number of vendors offering the technology increases. These vendors are primarily startup companies and small vendors that try to use the increasing amount of hype for their marketing benefit. A growing number of enterprises start to examine how the technology may fit within their business strategies, although most do not take action at this stage. Venture capitalists may be interested in selling some of the startups that they equipped with early funding. As problems with first-generation products become visible (e.g., emerging pest resistance in the Bt cotton regions [9, 10] and the latest success message of Huang et al. [11], often because the technology is pushed to its limits, negative publicity starts to push the technology into the Trough of Disillusionment, often the pertinent publications are pushed for negative statements beyond the limit of scientific rules (for example, Web services in 2002 and biometrics in 2003 and two example from the debate on non-target insects related to Bt crops: a) the case of the monarch butterfly [12] and b) Lovei et al. [13] giving false alarm for ladybirds and its prompt rebuttal by Antony Shelton et al. [14]).

- **2.4 Sliding into the Trough of Disillusionment.** Because the technology does not live up to enterprises' and the media's overinflated expectations, it is rapidly discredited. Some of the early trials end in highly publicized failures. Media interest wanes, except for a few cautionary tales. A significant amount of vendor consolidation and failure occurs. Later-stage investors may be interested in funding vendors during this phase because equity is fairly inexpensive after the "microbubble" at the Peak of Inflated Expectations has burst. However, amid the disillusionment, trials are ongoing and vendors are improving products based on early feedback regarding problems and issues. Some early adopters find some benefit in adopting the technology. For some slow-moving technologies (for example, biometrics), workable and cost-effective solutions emerge and provide value in niche domains, even while the technology remains in the Trough. The Trough of Disillusionment coincides with the "chasm" in Geoffrey Moore's classic book, "Crossing the Chasm" [15]. During this stage, vendors need to launch their products from a few early adopters to adoption by a majority of enterprises to begin the climb up the Slope of Enlightenment. There is no real parallel in the GM crop history, except that the differences in GM crop regulation and perception between the Americas and Europe caused a deep transatlantic divide [16].

- **2.5 Climbing the Slope of Enlightenment.** Focused experimentation and real-world experience by an increasingly diverse range of enterprises lead to a better understanding of the technology's applicability, risks and benefits. Vendors seek mezzanine or later-round funding for marketing and sales support to pull them-selves up the Slope. Second- and third-generation products are launched by the leading seed companies, and methodologies and tools are added to ease the development process, see the

sections under 1.2. The service component declines as a percentage of the sale. Technologically aggressive ("Type A") enterprises are relatively comfortable adopting the technology, and moderately aggressive ("Type B") enterprises start to investigate and pilot the technology. Conservative ("Type C") enterprises remain wary. At the beginning of the slope, the penetration often is significantly less than 5 percent of the potential market segment. This will grow to approximately 30 percent and more as the technology enters the Plateau of Enlightenment. Examples of more or less unexpected enhancements in science and risk assessment of transgenic crops come from a higher precision of gene transfer methods (see sections under 1.2.), also compare to the latest developments in resistance management with a clear success story this year [11].

- **2.6 Entering the Plateau of Productivity.** The Plateau represents the beginning of mainstream adoption, which began in the Americas much earlier from 2000 onwards, when the real-world benefits of the technology are demonstrated and accepted, see the consecutive reports on the world development of transgenic crops on www.isaaa.org. Technologies become increasingly embedded into solutions that increasingly are "out of the box," with decreasing service elements as the technology matures (example conservation tilling). The majority of Type B, then Type C, enterprises adopt the technology. As a high-profile technology matures, an "ecosystem" often evolves around it. The ecosystem supports multiple providers of products and services, and also a market for related products and services that extend or are based on the technology (for example, virtual private networks in 2003 or the growing market for suppliers of molecular laboratories or the growing market for electronic equipment for precision agriculture).

  The final height of the Plateau varies according to whether the technology is broadly applicable or benefits only a niche market, depending heavily on crop and region.

- **2.7 Post-Plateau.** As a technology achieves full maturity and supports thousands of enterprises and millions of users, producers and consumers, its hype typically disappears, as seen in the Americas. Only a few specialist magazines continue coverage of new aspects of implementing and maintaining the technology. Often there may be innovations around this technology that will follow their own Hype Cycles (new crop varieties on stress resistance, on bio-fortification, pharmaceutical crop lines etc.).

- **3.0 The Time-to-Maturity Assessment.** Technologies do not move at a uniform speed through the Hype Cycle. It often takes years for a technology to traverse the Hype Cycle — some technologies like GM crops may take decades, with considerable regional differences. There are three adoption speeds:

  "Fast-track" technologies go through the Hype Cycle within two to four years. This occurs when the performance curve inflects early in the life cycle of a technology. These technologies find themselves adopted without much fanfare, bypassing the Peak of Inflated Expectations and Trough of Disillusionment. Many enterprises are unaware of their sudden maturity and applicability, such as what has happened with instant messaging and Short Message Service.

It is interesting to note that the Showalter "hystories" on the introduction of most new technologies [5] report no real damage in their subsequent introductory phase, or the benefits were so overwhelming that the debate was soon fading away. This alone demonstrates clearly that it is the sociocultural environment strongly influencing the risk debate [17]. The most recent events seem to hint that Europe finally finds to a more de-contracted way of looking at GM crops: The new report of the Royal Society [18] tries to unite conventional and biotechnology approaches for the sake of making progress on agricultural management in developing countries:

- Past debates about agricultural technology have tended to involve different parties arguing for either advanced biotechnology including GM, improved conventional agricultural practice or low-input methods. We do not consider that these approaches are mutually exclusive: improvements to all systems require high-quality science. Global food insecurity is the product of a set of interrelated local problems of food production and consumption. The diversity of these problems needs to be reflected in the diversity of scientific approaches used to tackle them. Rather than focusing on particular scientific tools and techniques, the approaches should be evaluated in terms of their outcomes.

It might well be that we arrive sooner than expected from a period of disillusionment to an eventual understanding of the technology's relevance and role in a market or domain.

Innovation in Agriculture on all Levels will Speed up and Makes it a Necessity to Rethink Regulation Basically and Radically, most Often in the Direction of Lowering the Regulatory Hurdles

Unfortunately, regulatory legislation is in its nature static, needs a long time to be settled in international negotiations, and then, finally, settled and approved with an important number of signatory states as the Cartagena Protocol; therefore, it is nearly impossible to make the necessary changes based on good science. At the time of the establishment of the Cartagena Biosafety Protocol, the similarities between nontransgenic and transgenic organisms on the molecular level were not widely known, although properly published (see latest review with early publications [19]), and a correction about these grave errors (recently called by the author as "Genomic Misconception," publication in preparation) in concept is now nearly impossible – details in section GM- and Non-GM-Crop Differences Over-Estimated, the "Genomic Misconception". But the situation is not getting better: the accelerating speed of scientific progress and discoveries used for new (agricultural) technologies is breathtaking. A short overview is provided in the following sections.

New Biotechnology Approaches in Plant Breeding, Introduction In an early paper, Britt et al. give an overview on many molecular possibilities which will develop for new breeding successes [20], they address the current status of plant gene targeting and what is known about the associated plant DNA repair mechanisms. One of the greatest hurdle that plant biologists face in assigning gene function and in crop improvement is the lack of efficient and robust technologies to generate gene replacements or targeted gene knockouts. They also face an old problem in plant breeding summarized under the complex term of epigenetics [21, 22], a problem corrected in conventional plant breeding by careful and often tedious selection processes. Unfortunately, opponents abuse epigenetics as a seemingly new problem for genetic engineering [23], avoiding the mention of modern molecular insight and its ease to correct such problems in a more targeted way. It is clear that transgenesis will remain a solid technology for breeding, but new approaches will appear – as science is always open for progress and new breakthroughs. Here, we only mention shortly progress from another more holistic perspective of systems biology: the dynamics of Metabolomics [24], and also the growing speed of discovery in proteomics [25], techniques which will increasingly augment more common types of experimentation, especially as they provide the capacity of generating data sets that can be compared across studies and laboratories [26], and because quantitative proteomics data are generated with unprecedented sensitivity, accuracy, and reproducibility. There are many new biotechnologies enhancing the speed of achieving targeted breeding successes such as the high throughput marker finding technology [27, 28], only a few can be mentioned here:

***Cis*- and Intragenic Approaches** A new technology has now proven to be a successful strategy: As Romments describe it, cisgenetics is a welcome way of combining the benefits of traditional breeding with modern biotechnology. It is an understandable enthusiasm of the first researchers using this technology to emphasize the positive sides by also comparing to transgenesis as an "old-fashioned" method with its problems. But things are certainly not so easy: In sections Molecular Processes Similar in Natural Mutation and Transgenesis and Dissent Over Differences Between GM- and Non-GM Crops Causes Transatlantic Regulatory Divide, it is made clear that on the genomic level, particularly on the level of molecular processes, there is no difference between transgenic and nontransgenic crops (supported by an important body of scientific literature), and this is certainly also true to cisgenic and intragenic varieties. This is why it is questionable and based on false grounds to make claims that those new methods in transformation would be safer, as Giddings has made it clear in his letter [29], and his arguments against the views of [30–32] and later publications [33–35] could have been targeted as well: they try to demonstrate that the new cisgenics and intragenics are safer than transgenics, which is not

based on any facts, rather it is based on accepting without scientific scrutiny the negative public perception on transgenic crops. It is also wrong to use without clarification the term "alien genes" in view of confirmed and widely accepted universality of DNA and genomic structures.

However, there is nothing to say against the application of such new methods per se, as [33, 34] can demonstrate:

- The classical methods of alien gene transfer by traditional breeding yielded fruitful results. However, modern varieties demand a growing number of combined traits, for which pre-breeding methods with wild species are often needed. Introgression and translocation breeding require time consuming backcrosses and simultaneous selection steps to overcome linkage drag. Breeding of crops using the traditional sources of genetic variation by cisgenesis can speed up the whole process dramatically, along with usage of existing promising varieties. This is specifically the case with complex (allo)polyploids and with heterozygous, vegetative propagated crops. Therefore, we believe that cisgenesis is the basis of the second/ever green revolution needed in traditional plant breeding. For this goal to be achieved, exemption of the GM-regulation of cisgenes is needed.

Reverse Screening Methods: Tilling and Eco-Tilling Two rather independent publications [36, 37] with largely incongruent literature lists promote a new technology of finding useful genes within the genome of the crops involved: They both promote powerful reverse genetic strategies that allow the detection of induced point mutations in individuals of the mutagenized populations, can address the major challenge of linking sequence information to the biological function of genes, and can also identify novel variation for plant breeding [37]. Rigola et al. [36] develop reverse genetics approaches which rely on the detection of sequence alterations in target genes to identify allelic variants among mutant or natural populations. Current (pre-) screening methods such as *tilling* and *eco-tilling* are based on the detection of single base mismatches in heteroduplexes using endonucleases such as CEL 1. However, there are drawbacks in the use of endonucleases due to their relatively poor cleavage efficiency and exonuclease activity. Moreover, prescreening methods do not reveal information about the nature of sequence changes and their possible impact on gene function. Rigola et al. [36] present a *KeyPointTM* technology, a high-throughput mutation/polymorphism discovery technique based on massive parallel sequencing of target genes amplified from mutant or natural populations. Thus, *KeyPointTM* combines multidimensional pooling of large numbers of individual DNA samples and the use of sample identification tags ("sample barcoding") with next-generation sequencing technology. Rigola et al. [36] can demonstrate first successes in tomato breeding by identifying two mutants in the tomato eIF4E gene based on screening more than 3,000 M2 families in a single GS FLX sequencing run, and discovery of six haplotypes of tomato eIF4E gene by re-sequencing three amplicons in a subset of 92 tomato lines from the EU-SOL core collection. This technology will prove to be useful and does not need for its own breakthrough to refer to a scientifically unjustified critique of transgenesis. Whether the new technology will replace the transgenic "Amflora potato" has still to be proven by further scrutinizing of the results of the equivalent trait [38].

Zinc Finger Targeted Insertion of Transgenes Plant breeding has gone through dynamic developments, from marker-assisted breeding to transgenesis with steadily improved methods to the latest development of the Zink-finger enzyme-assisted targeted insertion of transgenes in complex organisms [39–42]. Zinc-finger nucleases (ZFNs) allow gene editing in live cells by inducing a targeted DNA double-strand break (DSB) at a specific genomic locus. However, strategies for characterizing the genome-wide specificity of ZFNs remain limited. According to [43], comprehensive mapping of ZFN activity in vivo will facilitate the broad application of these reagents in translational research.

The development toward more insertion precision and less genomic disturbance is so rapid that promoters of organic farming will see dwindling one of their pet arguments even more rapidly: Genomic disturbance of modern breeding is certainly less important and will even be negligible compared to the old breeding methods, still promoted stubbornly by the

organic plant breeding community [44]: It is very likely that the transcriptomic disturbances will be even smaller in future – compared to the clumsy and tedious methods of conventional breeding, see also the latest developments in sections TALEs: Transformation Method Transcription Activator-like Family of Type III Effectors and Precision Engineering Through DNE Meganucleases below.

TALEs: Transformation Method Transcription Activator-like Family of Type III Effectors The generation of double-strand DNA breaks (DSBs) promotes homologous recombination in eukaryotes and can facilitate gene targeting, additions, deletions, and inactivation. Zinc-finger nucleases have been used to generate DSBs and subsequently for genome editing, but with low efficiency and reproducibility. In contrast, the transcription activator-like family of type III effectors (TALEs) contains a central domain of tandem repeats that could be engineered to bind specific DNA targets. The new method is capable of generating site-specific DNS Breaks and has great potential for site-specific genome modification in plants and eukaryotes in general [45]. See also comments on the newswire CNBS [46] on the discovery:

- Dr. Mahfouz has developed a "repair tool" (molecular scissors) made out of protein that does two things: it finds the exact place on the genome where it is to be cut using a genetic "postcode" and then deletes, adds or edits the gene with great accuracy and precision.

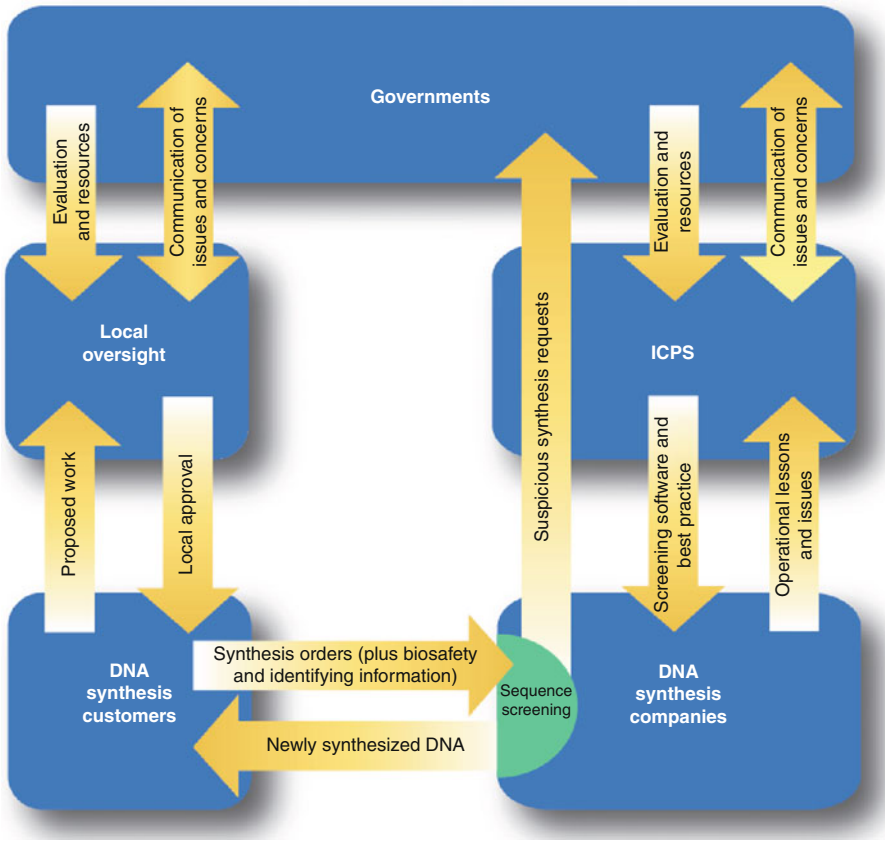
  Dr. Mahfouz's work has the potential for much broader applications including human health. This new technology could enhance the technique that may be used to substitute "good" genes for bad, or to cut out or silence the defective genes that cause disease.

  Commenting on the research, KAUST Provost Stefan Catsicas saw the technology as a scientific breakthrough and, if the patent is eventually successful, having potentially promising revenues. Dr. Nina Fedoroff, Professor of the Life Sciences at Penn State University, said the Mahfouz paper "shows the practicability of creating DNA-cutting enzymes tailored to cut a desired target sequence with very high specificity. This is an excellent step forward toward creating very specific genetic improvements in crop plants, while avoiding the potential risks many are concerned about with more conventional genetic modification strategies. Moreover, the paper gives the first evidence that this particular strategy will work in plants." Professor Fedoroff is "delighted to see such cutting-edge contributions emerging from a university as young as KAUST!".

Precision Engineering Through DNE Meganucleases Engineered DNE meganucleases can be used for cloning and molecular analysis purposes in much the same ways as conventional restriction enzymes. The important difference, of course, is that meganucleases recognize much rarer DNA sequences than restriction enzymes. This makes them particularly well suited to the manipulation of extremely large DNA sequences such as intact genomes. Importantly, DNE meganucleases cleave to leave four base pair 3' overhangs suitable for "sticky-end" cloning. The first application with a new tool called Directed Nuclease Editor™ in plant breeding by Bayer Crop Science http://www.precisionbiosciences.com/ seems promising: The meganucleases have been first used to do precision work in human gene therapy, but an outlook into various other applications was announced as early as 2003 [47–49].

Synthetic Biology In some 150 laboratories, synthetic biology is intensively researched, and it seems clear that the future will bring here some unexpected revolutions: A new field, synthetic biology, is emerging on the basis of these experiments [50], where chemistry mimics biological processes as complicated as Darwinian evolution. According to [51], the emerging field of synthetic biology is generating insatiable demands for synthetic genes, which far exceed existing gene synthesis capabilities. Tian et al. claim that technologies and trends potentially will lead to breakthroughs in the development of accurate, low-cost, and high-throughput gene synthesis technology – the capability of generating unlimited supplies of DNA molecules of any sequence or size will transform biomedical and any biotechnology research in the near future. And, according to [52], already in 1998 the redesigning of nucleic acids has been judged in an optimistic way, this was confirmed in an important Nature review in 2005 [53].

The real breakthrough came with the synthesis of an organism including its reproduction, achieved after



GM Crop Risk Debate, Science and Socioeconomics. Figure 2

Our framework calls for the immediate and systematic implementation of a tiered DNA synthesis order screening process. To promote and establish accountability, individuals who place orders for DNA synthesis would be required to identify themselves, their home organization, and all relevant biosafety information. Next, individual companies would use validated software tools to check synthesis orders against a set of select agents or sequences to help ensure regulatory compliance and flag synthesis orders for further review. Finally, DNA synthesis and synthetic biology companies would work together through the ICPS, and interface with appropriate government agencies (worldwide), to rapidly and continually improve the underlying technologies used to screen orders and identify potentially dangerous sequences, as well as develop a clearly defined process to report behavior that falls outside of the agreed-upon guidelines. ICPS, International Consortium for Polynucleotide Synthesis (From [58])

years of research and a firm belief in success, typical of the senior author of the mega project still continuing, [54–57].

A pragmatic view of a new regulatory scheme answering the new biosafety tasks of synthetic biology is proposed by [58] (Fig. 2):

This kind of new regulatory approach will be necessary in order to avoid unnecessary hindering of research progress in synthetic biology, a demand supported with other innovative suggestions for interactive procedures [59]. Another balanced view [60] demonstrates also the new risks arising from synthetic organisms and the accidental (or purposeful) release in the environment. As always, the ethical awareness and behavior has to be developed further, agreeing with [61] not in a way which gives forfeit power to social sciences. What we really need is a new interfaculty, interdisciplinary or, even better, transdisciplinary discursive scheme as proposed in sections Long Term Discourse and Decision Making

Processes and The Second Generation Systems Approach as a New Decision Making Process.

What happened some 35 years ago in the US National Institute of Health in the words of Henry I. Miller [62] should be a warning.

> Thirty-five years ago, the US National Institutes of Health adopted overly risk-averse guidelines for research using recombinant DNA, or "genetic engineering," techniques. Those guidelines, based on what has proved to be an idiosyncratic and largely invalid set of assumptions, sent a powerful message that scientists and the federal government were taking seriously speculative, exaggerated risk scenarios – a message that has afflicted the technology's development worldwide ever since.

A final remark: In a way, the artificial altering of genes producing Bt toxins can, strictly spoken, also be summarized under synthetic biology since the specifically altered Bt toxins in order to facilitate resistance management of Bt crops: Bruce Tabashnik, who works on problem solving programs for Bt crops with field research and new concepts of resistance management [63]: Relative to native toxins, the potency of modified toxins was >350-fold higher against resistant strains of *Plutella xylostella* and *Ostrinia nubilalis*. Previous results suggested that the modified toxins would be effective only if resistance was linked with mutations in genes encoding toxin-binding cadherin proteins [64]. Tabashnik et al. report evidence from five major crop pests refuting the Soberon hypothesis.

Illusions and Realities on Educational Effects in the Debate, the Dialogue Between Science and the Public

There is no doubt that there is hope and need to simply start and/or maintain an open dialogue between major stakeholders among young scientists, politicians, industry, and society [65], although there are many obstacles such as asymmetric relationships among the partners, which can render the discourse complex and unpredictable. And it is uncontested here that education on all school levels has its justified place; this has again been shown with empirical results from Spain [66, 67]. Gensuisse should also be mentioned here with educational activities in schools and a popular open day of Genetics in major Swiss cities organized by researchers and institutes every year [68]. And education on biotechnology in the developing world is especially important, if done in a participative way, and with proper ramifications in all institutions of communication, science, and regulation: In April 2007, biosafety and biotechnology scientists, regulators, educators, and communicators from Kenya, Tanzania, and Uganda met to examine the status and needs of biosafety training and educational programs in East Africa [69].

Thus, educational efforts on all levels are not in vain, and deplorably there are too few academic institutions active in biotechnology education [70]. The structure of the debate has shifted: Today, the GM crop debate is steered by scientific *and* pseudoscientific arguments. And this also includes an element of hope for the pro-scene: Slowly but surely the pseudoscientific arguments are fading away for the opponents, since there is no serious incident known despite the fact that millions of hectares are grown with GM crops worldwide [71].

There is a widespread mistrust against new technologies where everybody feels it will change their own life, and this often happens in a phase where the benefits are not yet clearly visible, especially for the consumers/users. But it is not correct to reduce those difficulties to an exclusive criticism of the so-called deficit model [72–74] where the people just have to be educated and then they would refrain from negative emotions. A question mark on the exclusive use of the "deficit model" is justified, but surprising conclusions emerge from the above-mentioned critics themselves: They do not discard altogether the traditional deficit model, rather they propose to combine it with the *contextual approach*, thus emphasizing the complex and interacting nature of the knowledge-attitude interface. This highlights the sophistication and value of lay understandings of science that can exist in the absence of formal scientific knowledge [75, 76]. Surprisingly, positive are results of polls which are conducted by Philip Aerni with more closeness to the real life and careful avoiding of polling mistakes [77], the study concludes:

> The results of our discrete choice analysis show that Swiss consumers treat GM foods just like any other type of novel food. We conclude from our findings that consumers tend to appreciate transparency

> and freedom of choice even if one of the offered product types is labeled as containing a genetically modified ingredient. Retailers should allow consumers to make their own choice and accept the fact that not all people appear to be afraid of GM food. [77]

There is growing consensus that scientific knowledge extends beyond the simple learning of "facts" that can be straightforwardly defined and measured [78]. From this perspective, privileging formal scientific knowledge as the sole basis of rational preference formation leads us to overlook other knowledge domains that may be equal or even more important determinants of attitudes toward science.

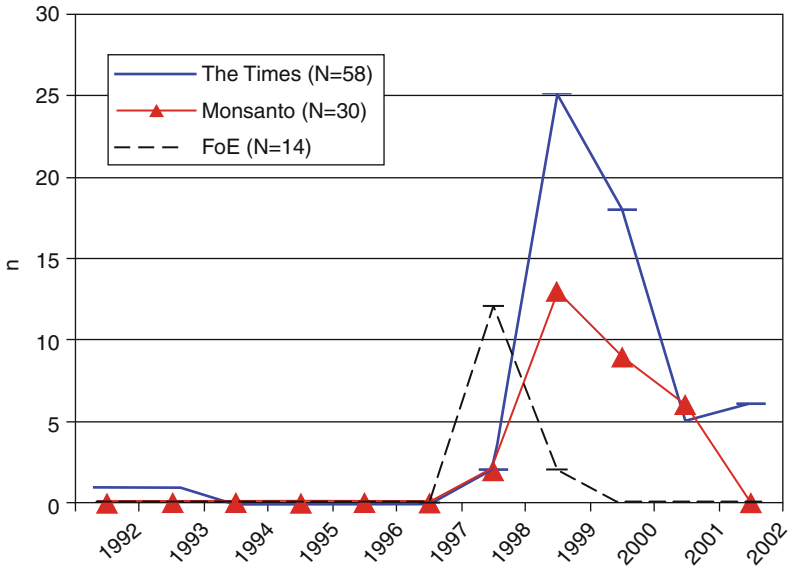
These insights have been condensed into a feasible discursive method of the *Systems Approach* initiated by Churchman [79] and refined by Rittel et al. [80–82]. Details on the methodology are given under sections Long Term Discourse and Decision Making Processes and The Second Generation Systems Approach as a New Decision Making Process, where the *solutions* are discussed.

It is an illusion to solve ill-fated GM-disputes by just adding social and cultural aspects, or that the dispute should, so to say, start from the other end of the controversy ignoring the biosafety science [83] or even worse to primarily appeal to feelings and emotions of the public and indulge in entertaining but ultimately meaningless discussions in order to catch the interest of the public – we should not mimic the strategy of the protest corporations. That said, this does not mean that sociocultural aspects including emotions should be neglected – even the boulevard press sends out strong signals for learning processes. Vaughan's [84] plea is that regulatory officials should engage in an interactive process of information and opinion exchange that is reasonable and effective within vastly different socioeconomic and cultural contexts, This is often a challenge to government employees concentrating on office work routine. Patricia Osseweijer [85, 86] offers an interesting compromise: a mix of science, ethics, and emotions with her "Three E-Model" Entertainment (getting attention), Emotion (identification), and Education (information and skills for [future] decision making). It has been developed on the basis of long-term experience and observation of public communication by individuals in the Department of Biotechnology of the Delft University of Technology [87, 88].

Despite all possible refinements and enhancements in the dialogue with the public, we should not underestimate the negative role of the opponents of genetic engineering in plant breeding organized as professional protest corporations, see section The Costs and Loss Benefits of Overregulation.

How the Internet is Influencing the Debate The Internet as a worldwide literacy practice environment is still underestimated, nevertheless it has created a new situation in communication, providing a new dynamic field for research and knowledge accumulation [89]. It has created an Internet-based debate culture with all its ramifications from classic email over blogs and better organized social media to twitter and this not only in nanotechnology [90], but also in other research realms and E-business [91]. The evolution in this kind of debate is still going on with unprecedented dynamics and is not yet fully understood in all its consequences [92], [93], and [94]. The hope is that easier communication through the Internet will invite a *collaborative* instead of *confronting* modus [95]. Some advice on how to behave in chats and blog debates on the Internet might be useful [96]; compare a list of useful websites and databases on biosafety by DeGrassi et al. [97] and [98]. A list of pertinent websites can be expanded ad libitum, the present state of error of 2011, with all the personal bias in [99].

Informatics and the new ease to access huge amounts of scientific information on the Internet causes a democratization effect on the science debate. But this can only then lead to positive developments if the new flood of information is also well organized and provided people make serious efforts to analyze the available information, so that our understanding of complex scientific knowledge can indeed be improved. As Janetzko (2008) shows, it is not enough to make use of the most common search machines, only professionally organized searches and databases on scientific literature can help and create some limited reliability and sustainability of scientific knowledge. And: clearly, the usual citation clusters among opinion-buddies will not suffice. And it should be emphasized: Electronic ease does not replace the tough job of scholarly reading and



GM Crop Risk Debate, Science and Socioeconomics. Figure 3
Web site pages addressing the "Frankenfood" and "Frankenstein food" issues at Monsanto, the *Times*, and the Friends of the Earth Web sites. jcmc.indiana.edu/vol8/issue4/hellsten.html

understanding. It will be a difficult task for the future to divide up clever knowledge accumulation and genuine thinking work among active scientists. A caveat already signaled by Seneca: Thoughtful Action creates more wisdom than knowledge accumulation, can be interpreted related to social electronic networking in two ways: On one side, the immense intensification of social networking via the Internet creates among other things a new possibility for post-publication reviewing and filtering out the really relevant publications and ideas. On the other hand, it hinders systematically the deepening of your own knowledge in an individual way, and be it only by reading every year a dozen or two really relevant book publications.

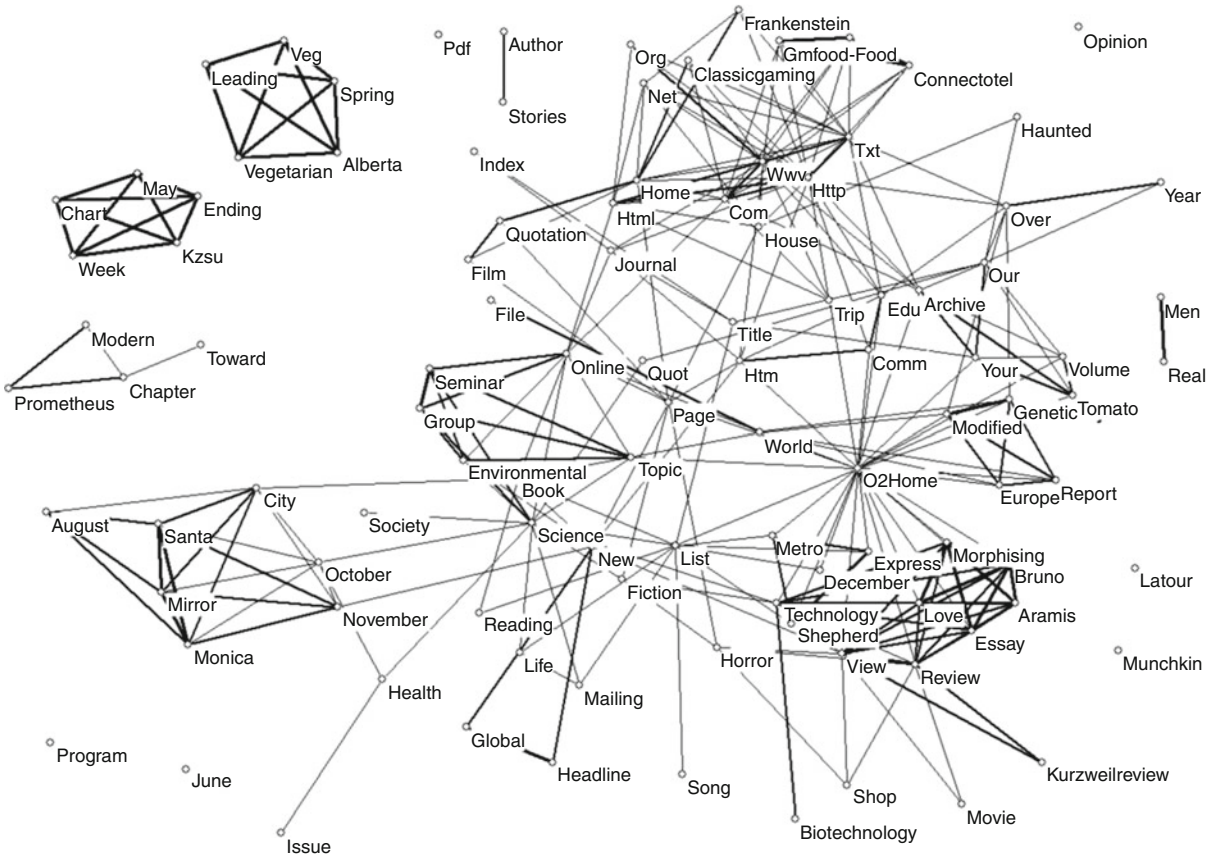
This major shift from paper to electronics is also creating new methods of *quantitative* analysis of scientific work: see the Scientometrics Wikipedia: http://en.wikipedia.org/wiki/Scientometrics. Actually, this newly emerging science can provide with caveats and insights into changes in research priorities, reveal citation habits, evaluate journals with new scales, etc. [100–103]. A typical example is given in the analysis of the coming and going of the Frankenfood myth [104], with a somewhat surprisingly early and sharp peak of appearances of the word Frankenfood in websites for 1998, followed by a sharp decline to virtually zero 2 years later (Fig. 3).

This figure is confirmed in [104] with the following statements and figures (Figs. 4 and 5):

The comments in [104]:

- Our interpretation of these results is as follows: the decline of the organizing power of the metaphor was rapid in 1999 and 2000 when the metaphors of 'Frankenfood' and 'Frankenstein food' began to be outdated. Due to its generalized meaning, the metaphor was used increasingly across domains and therefore lost its domain-specificity and the ability to organize distinctions among domains. This might also explain why the NGOs stopped using the metaphor in 2000 (HELLSTEN, 2003). From [104]

Scientometrics can do much more, [105] have shown the potential of a sophisticated statistical analysis combined with modeling of community interactions in the web: Besides tracking just the description-to-acquisition behavior of users, scientometrics can do much more by longer observation periods which offers the chance to make richer inferences about both group and individual user intentions – trends of intruding into human behavior and making



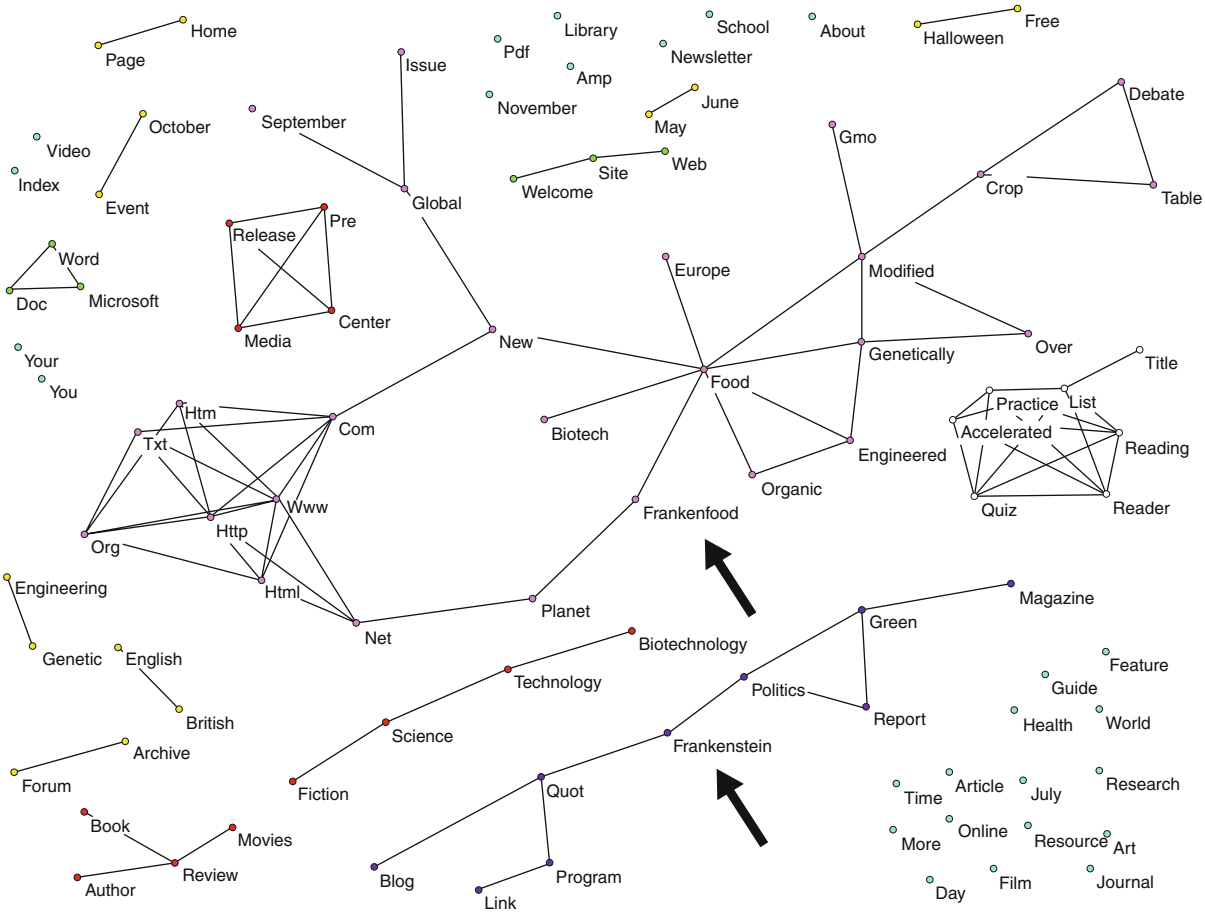
GM Crop Risk Debate, Science and Socioeconomics. Figure 4
The cosine map of 107 words used more than once in the 205 documents on Frankenfoods in 1999 (cosine $\geq$ 0.1) (From [104])

conclusions, which are actually beyond Orwell's imagination. Yet we should have no illusions, since a lot of work and application is already going on in the marketing and advertisement scene, which has also an often manifested interest in knowledge accumulation methods [106, 107]. It is somehow amazing to realize that the academic world in most fields of specialization have not yet reached the realms of professional knowledge accumulation and consolidation – not to speak about an efficient way of reaching out from knowledge accumulation to efficient development of new technology. Scientometrics would have the potential to get instrumentalized in research and development, with some good chance to be used also in new peer review processes.

A *qualitative* evaluation of science should involve additional elements – see below under peer review in the section Developments in Risk Handling of GM Crops on regulation.

Deplorably, important networks are often only known in specific reader clusters, these awareness gaps should be minimized. We need knowledge exchange, jumping over geographical and ideology fences.

Science Education and New Developments on the Internet In a successful initiative, Ron LaPorte and his group "Supercourse" started in 2002 [108] a new educational Internet-based system: In his view, Journals do not have an exclusive "right" to science. A publication and a scientific presentation do virtually the same thing – they share scientific knowledge. Publication and presentation have been separate but could "morph" into a single entity. This metamorphosis is taking place and is driven by a juggernaut called



GM Crop Risk Debate, Science and Socioeconomics. Figure 5
The cosine map of 100 words used more than 31 times in the 6,101 documents on Frankenfoods in 2003 (cosine $\geq$ 0.1) (From [104])

PowerPoint, Microsoft's graphics and slide presentation software, and today enriched with more media from Twitter over YouTube to all the numerous blog systems, networking enhanced with RSS, etc. More on the Supercourse program in [109–113], also in connection with the Bibliotheca Alexandrina in Egypt: [114] Another possibility on a well-organized collection of Powerpoint slides is offered for free by the University of California by Peggy Lemaux and Barbara Alonso, University of California http://ucbiotech.org/resources/slide_archive/index.html. A series of over 100 slideshows is offered by the bibliography of the author; new slide shows are continuously added, they can be downloaded from [115]. An important new development started 2002 at the Bibliotheca Alexandrina, where a new world center of electronic knowledge is emerging, which is based on thoughtful new structures [116].

On Biosafety Education Biosafety is today a permanent topic on local, national, and international level, and basically, it is good to see educational activity. As demonstrated in this contribution, the topic of biosafety is highly controversial, and so are the views on the various educational activities. The most blatant misunderstandings in biosafety education stem again from the "Genomic Misconception," which forces authors seemingly to focus on transgenic crops alone, which is scientifically unacceptable as we will see in section GM- and Non-GM-crop Differences

Over-estimated, the "Genomic Misconception". A symptomatic example on the enumeration of risks related to transgenic crops is given by Craig et al. [117]: All risks duly mentioned can be attributed just as well to conventional crops. The only difference between modern and conventional breeds can be found in risk mitigation, which is much easier in the case of the transgenic crops. Here, just two recent examples related to the successful prevention of upcoming resistant pest insects (a problem arising in all kinds of agricultural management systems): [63] and [11]. It is deplorable, that most biosafety education is still based on the erroneous "Genomic Misconception," which results automatically into a biosafety risk view focusing on the process of transgenesis instead of working on a product-oriented basis. More about the "Genomic Misconception" is discussed in section GM- and Non-GM-crop Differences Over-estimated, the "Genomic Misconception".

Proposal for a Website of Websites There are simply too many websites (see ASK-FORCE Organization and Related Websites) and not enough coordination, so there is a need for networking structures among the most important websites, a *network of networks* with all the fancy new buttons available like RRS, etc. There should be a place where people see with one glance on the first page what news they can expect on various important sites. It should also not be difficult to add possibilities for an individual choice.

Those website connection activities need professional support with some secretarial/managerial help. We must work out ways in which the broad public can easily reach rebuttals on all the myths, facts, and benefits in the debate on green biotechnology. It will not be difficult to establish a platform for a better communication among the most important websites – in the field of agricultural biotechnology, there are a few very successful ones, but this is not the whole task. We need to look deeper into the theory of networks in order to be really successful; comprehensive reviews demonstrate how complex the networking task really is [118, 119].

As of now, this is just an idea and needs to be discussed with Internet and website specialists. One of the main difficulties will be to establish permanent existence, this is why it would be best to use structures having proofed long years' activities and assured permanence, such as ISAAA, the International Service for the acquisition of Agri-Biotech Applications, www.isaaa.org. After all, the leading webmasters and coordinators agree that it is time to *enhance collaboration through better communication*, see section ASK-FORCE Organization and Related Websites. ASK-FORCE. The task on uniting the most relevant websites and blogs should not be underestimated, see the list already given above [99].

Developments in Risk Handling of GM Crops

General Views on the Dialogue Related to Regulation of GM Crops

The dialogue between scientists and regulators is very complex, as accurately described by Saner [120]. This should be a reminder that it is not about facts alone:

- It should be clear without explanation that each and every rational decision is a combination of facts and values – a decision requires judgment. The agents of judgment are, of course, people, and this leads us to an entirely different interface – that between scientists and policy-makers.

We should keep this in mind when we concentrate here on the *science* of GM crop regulation. See also the analysis of the debate in The General Strategic Situation of the Debate About Green Biotechnology Today. These philosophical thoughts of Saner are at the basis of the discursive methodology for complex decision-making processes, [121–123]. For details, see below in this contribution in sections Long Term Discourse and Decision Making Processes and The Second Generation Systems Approach as a New Decision Making Process.

A valid overview on the regulatory science and traceability related to GM crops has been published by Gasson and Burke [124, 125], there is no intention to repeat these reviews.

Biotechnology and Economics

How Economics Are Influencing the GM Crop Debate The example of the Flavr Savr Tomato demonstrates that in earlier times, even in Europe, GM food was well received, but several factors just made it clear

that economic success was missing [8, 126–128]. And regulation of this pioneer work needs to get a new look; with modern screening methods, the gene silencing on the molecular level revealed some surprises [129].

Economics play a very important role in the process of technology acceptance: This can be illustrated with the present day feed import situation in Europe. First it should be mentioned, that it is the trade policy of Europe still going the wrong way, which causes a lot of difficulties in the transatlantic dialogue: As Graff et al. [130] explain:

> ▶ European policies blocking genetically engineered crops are conventionally attributed to the concerns of European consumers, but they can be attributed to the self-interests of European industry and farmers as well. Biotech policies maintained in the name of consumer interests are helping European chemical firms to slow their losses in the global crop protection market and are helping European farmers differentiate their conventional crops on environmental and safety grounds, maintain their agricultural subsidies and win new non-tariff trade protections.

The recent development in feed supplies, see Lawrence in *The Guardian* [131], in the EU provides argument, and the reports and letters below give excellent examples:

- Food Chain Dossier 2009: http://www.botanischergarten.ch/Feed/Food-Feed-Chain-Dossier-20090616.pdf
- DG AGRI feed report: http://www.botanischergarten.ch/Feed/EC-DG-AGRI-Rep-feed-situation-UnapprovedGMOs-200709.pdf
- EU Report on Pipeline: http://www.botanischergarten.ch/Feed/Stein-EU-Report-GMO pipeline-LLP-2009.pdf
- Letter to the President of the EU Commission Barroso: http://www.botanischergarten.ch/Feed/Letter-big-Producers-Tolerance-Value-Barroso-20090624.pdf

Strict labeling and thus a discrimination of European meat from animals fed with GM crops will soon be impossible as a political goal due to *economic* reasons – as it is also scientifically not justifiable [132, 133].

An interesting thesis with economic arguments is promoted by Paarlberg [134]: Today, Africa's production of GM crops is exported mainly to other African countries, and this might go on this way in the coming years, so the reasoning that Africans would destroy export opportunities to Europe by developing their own GM crops is not really convincing. But in reality, there is growing concern: Commercial fear over potential loss of export sales to Europe and East Asia is also a reason for mounting pressure on biosafety approvals in developing countries. Consumer misgivings toward GM food in rich countries combined with restrictive import and labeling policies are prompting GM-free agricultural production in developing countries. The long-term costs of these negative trends could be enormous [135]. Good arguments for this view are produced with lots of facts on economics and negative labeling effects of European developed countries, published by Gruère et al. [136–138]:

> ▶ In this context, the marketing decision of avoiding GM ingredients in food items rapidly became a quality attribute employed in the competition among the retails chains of Europe, Japan and South Korea. A report by the international NGO, Greenpeace, which has encouraged companies to adopt GMfree policies, provides evidence of the widespread adoption of such practices in Europe [139] as follows:
>
> - Fourteen of these retailers have a policy of not selling GM-branded products under their company name for all European countries. These include Carrefour, Auchan, Sainsbury's, Safeway, Marks & Spencer, Coop Switzerland, Coop Italia, Migros, Big Food Group, Somerfield, Morrison's, Kesko, Boots, and Co-op UK.
> - Seven of these retailers have a non-GM policy for their own branded products for their main markets (mainly in their home countries). These include Tesco, Rewe, Metro Group, Casino, Edeka, Schwarz group, Tengelmann).
> - Out of the top 30 European food and drink producers, 22 have a non-GM commitment in Europe, including Nestle, Unilever, Coca Cola, Diageo, Kraft Foods (Altria), Masterfoods (Mars), Heineken, Barilla, Carlsberg, Dr. Oetker, Arla Foods, InBev (Interbrew), Heinz, Chiquita, Cirio del Monte, Orkla, Ferrero, Northern Foods, Eckes Granini, Bonduelle, Kellogg and McCain.

- Thirteen of these 22 multinationals have a company-wide non-GM policy beyond Europe. These include Diageo, Heineken, Barilla, Carlsberg, Arla Foods, Dr. Oetker, Chiquita, Cirio del Monte, Orkla, Ferrero, Northern Foods, Eckes Granini, and Bonduelle [138].

Some companies even go beyond banning processed products derived from GM ingredients to include requirements on GM-free animal feed in animal products. Virtually all supermarkets sell only poultry fed with non-GM feeds, whereas the policies for dairy products, beef, and pork vary. The usual crude Greenpeace mix of facts and interpretation helped efficiently to push the companies for the European market to go GM crop free [139, 140]. The simple fact of labeling allows opponent NGOs to drive a polemic campaign of pompous "contamination" reports, thus delivering junk science "evidence" that there is some risk involved in the numerous events of minute admixtures of transgenes traces.

In India, there is a clear positive trend visible since some years after some difficulties in the beginning because local traits had to be created for the many Indian regions and also because there was right from the beginning a black market with illegal cotton traits developing (which often did better commercially than the legal ones. Presently, there are 38 traits of GM cotton in India [141].

The whole complex story has been recently summarized by [142]:

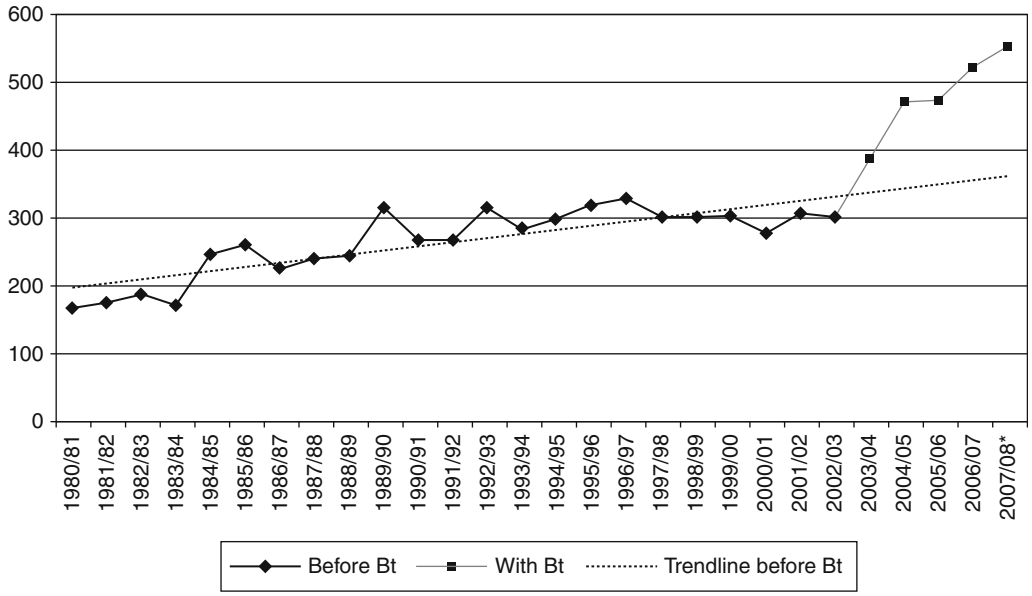
- On average, Bt-adopting farmers **realize pesticide reductions of roughly 40%, and yield advantages of 30-40%. Profit gains are at a magnitude of US $60 per acre**. These benefits have been sustainable over time. Farmers' satisfaction is reflected in a high willingness to pay for Bt seeds. Nonetheless, in 2006 Indian state governments decided to establish price caps at levels much lower than what companies had charged before. This intervention has further increased farmers' profits, but the impact on aggregate Bt adoption was relatively small. Price controls might have negative long-term implications, as they can severely hamper private sector incentives to invest in new technology. [142]

At the end of the day the profitability of Bt cotton is now uncontested, see comments of Müller-Jung Frankfurter Allgemeine: [143]

Also the old wrong connection between suicides of Indian farmers and the introduction of GM cotton in India has been thoroughly falsified [144, 145]. This does not hinder activists like Vandana Shiva from continuing with cheap propaganda linking GM crops with the sad tradition of farmers' suicides in India, which started decades before the introduction of GM crops and beginning activities of multinational seed companies. Here are two of the many graphs from [145] (Figs. 6 and 7):

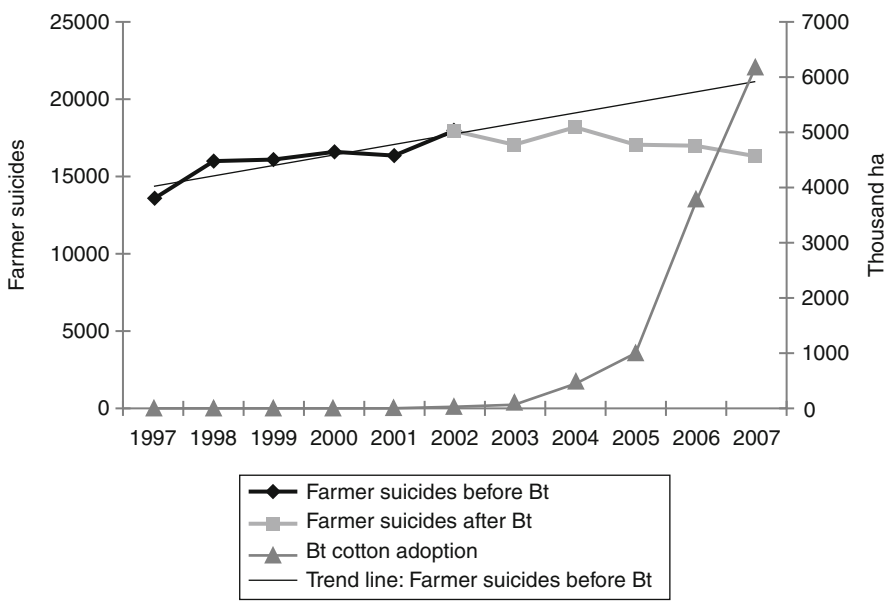
- **Abstract.** Bt cotton is accused of being responsible for an increase of farmer suicides in India. In this article, we provide a comprehensive review of evidence on Bt cotton and farmer suicides. Available data show no evidence of a 'resurgence' of farmer suicides. Moreover, Bt cotton technology has been very effective overall in India. Nevertheless, in specific districts and years, Bt cotton may have indirectly contributed to farmer indebtedness, leading to suicides, but its failure was mainly the result of the context or environment in which it was planted [145].

- **From the discussions.** The absence of irrigation systems in drought-prone areas (especially in Maharashtra), combined with specialisation in high-cost crops, low market and support prices, and the absence or failure of the credit system, is a clear recipe for failure. It is possible, therefore, that under the conditions in which it was introduced, Bt cotton, an expensive technology that has been poorly explained, often misused and initially available in only a few varieties, might have played a role in the overall indebtedness of certain farmers in some of the suicide-prone areas of these two states, particularly in its initial years. But none of these possible links has been explicitly demonstrated with a sufficiently robust analysis. One implication of this study is the critical need to distinguish the effect of Bt cotton as a technology from the context in which it was introduced. Revealed preferences based on farmer adoption rates and official or unofficial data all point toward the overall success it has had in controlling pest damage and therefore raising average yields in India. In particular, the increasing adoption rate in two suicide-prone states, Andhra Pradesh and Maharashtra, indicates that farmers in these states found this technology economically beneficial.



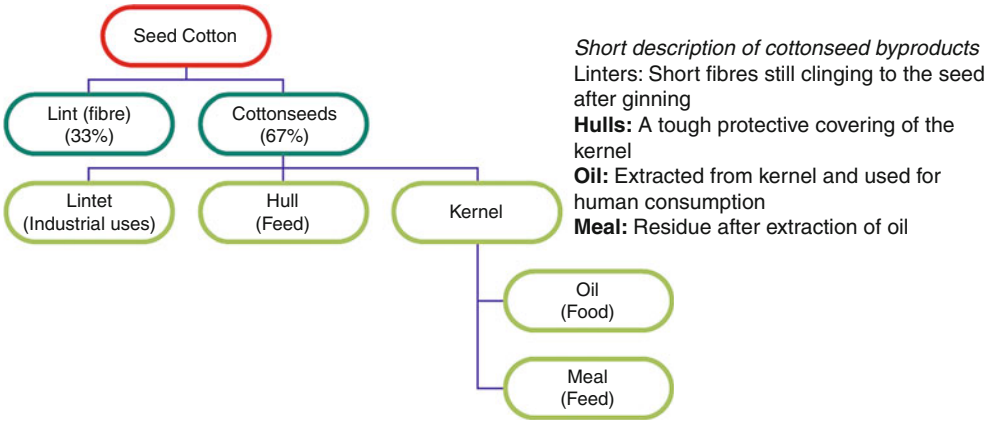
GM Crop Risk Debate, Science and Socioeconomics. Figure 6

Average cotton yields in India (kg/ha), 1980–2007 (Source: International Cotton Advisory Committee (2008). Note: Data for 2007/2008 is an estimate. From [145])



GM Crop Risk Debate, Science and Socioeconomics. Figure 7

Farmer suicides and Bt cotton area in India, 1997–2007 (Source: Combined data from Table 1 and Table 2. From [145])



GM Crop Risk Debate, Science and Socioeconomics. Figure 8
Cotton seed byproducts (From [148])

In contrast, marketing constraints and institutional issues may have played a significant role. Our analysis suggests the need for a better extension system, more controlled seed marketing system, anti-fraud enforcement and better information dissemination among farmers in all regions, before the introduction of any costly new technologies like Bt cotton. Farmers should also be encouraged to diversify their farming and non-farming activities to spread the risks they may incur.

The second implication is that, as farmer suicides are not new or specific to recent cases or to the introduction of Bt cotton, they point toward the failure of the socioeconomic environment and institutional settings in rural dry areas of India. This has nothing to do with cotton or the use of new technology and would suggest many potential policy changes. In several states, such as Karnataka and Andhra Pradesh, some policy changes have already been proposed. Lastly, much more and better federal and state investment could help prevent the 80 percent or more other cases of suicides.

This does not hinder activists like Vandana Shiva from proclaiming Indian farmers' suicides to be the fault of international corporations: [146] and lately also at a Barilla webinar July 20, 2011 in Milano: http://www.barillacfn.com/en/biotecnologie, she also does not shy away from connecting the sad tradition of farmers suicides in India with the emergence of GM crops, despite hard facts as demonstrated above. In the same picture you can see her pompous literature list she gives in her curriculum of "over 300 scientific publications in important journals" – a quick test in the comprehensive database of the Web of Knowledge http://apps.isiknowledge.com/ reveals some 47 papers, most of them in less important journals and magazines – so much about her scientific achievements.

A new perspective is open since 2006 for the production of cotton seed (oil for human consumption), seed meal for feed, made possible thanks to the detoxification (gossypol) successfully done by modern breeding including genetic engineering [147], see the latest summary on the matter (Fig. 8) [148]:

This latest development will open new doors for the cotton production and marketing.

The Political Economy of Biosafety Regulation in Agriculture An in-depth analysis of how politics is influenced by multiple factors of discursive processes, influenced by economics, has been developed by Graff et al. [149]. They are giving highly differentiated insights in the network of self-interests with some interesting examples of units influencing in their own interest the debate on GM crops: opponents of genetically engineered crops and also industrial units fearing losses in pesticide sales. Often these important socioeconomic elements in the regulatory debate are neglected and it seems to be difficult for all the regulatory analysis to bring together socioeconomic *and* molecular plant breeding aspects.

- This article develops a political-economy framework to analyze the formation of agricultural biotechnology policies. Going beyond accounts, that largely attribute differences between US and European regulatory environments to consumer attitudes, we consider the impact of what amounts to a Schumpeterian process of "creative destruction" across the entire range of relevant economic sectors and interests. **The analysis suggests that in Europe and in some developing countries a "strange bedfellows" constellation of concentrated economic interests (including incumbent agrochemical manufacturers, certain farm groups, and environmental protest activists)** act in rational self-interest to negatively characterize GM technology in the public arena and to seek regulations that block or slow its introduction. In contrast, those interests most likely to experience welfare gains from biotechnology are the more diffused and less informed – including consumers and small farmers. The most profound implications of overregulation of agricultural biotechnology are (1) delays in the global diffusion of proven technologies, resulting in a lower rate of growth in the global food supply and higher food prices, and (2) disincentives for investing in further R&D, resulting in a slowdown in innovation of second generation technologies anticipated to introduce broad consumer and environmental benefits." [149]

Ayal and Hochman [150], started in some intricate experimental setups working on the cognitive processes underlying choice behavior. With a mix of behavioral actions combined with opinion polls they found that people *do not rely on limited arguments only, but tend to integrate all acquired information into their choice processes*. This could explain the delay in such opinion finding and decision-making processes influencing politics over years, described in the Gartner hype cycles, see The General Strategic Situation of the Debate About Green Biotechnology Today.

Although this would be an epic theme, we shall concentrate here more on the debate of the *Science* of regulation and some discursive elements.

Brazil, A Case Where Politics Positively Influences the Development and Adoption of GM Crops

Studying the biosafety law of Brazil, the similarities with the European legislation cannot be overlooked: Both legislations are process-oriented and obey strict rules on biosafety assessment, including field experimentation:

A closer look at the Brazilian legislation [151] shows the similarities to the European legislation.

- Article 3. Under this Law, it shall be considered:
  V – genetically modified organism – GMOs: an organism the genetic material of which – DNA/RNA has been modified by any genetic engineering technique;

And compare some exclusion rules, typically reducing the safety assessments strictly to the process of genetic engineering.

- Article 4. This Law is not applicable when a genetic modification results from the following techniques, provided they do not imply in using a GMO as the receiver or donator:
  I – mutagenesis;
  I – the formation and use of animal hybridome somatic cells;
  III – cellular fusion, including plant cells protoplasm, which can be produced from traditional culture methods;
  IV – the self-cloning of naturally processed non-pathogenic organisms.

The same is the case in the European law:

[152], in the introduction the definition of GMOs is given:

- In order to protect human and animal health, food and feed consisting of, containing or produced from genetically modified organisms (hereinafter referred to as genetically modified food and feed) should undergo a safety assessment through a Community procedure before being placed on the market within the Community.

The intention of this "exclusive" definition is clear in this European Law: it should be restricted to GMOs which are wrongly defined as "genetically modified crops," a scientifically questionable denomination, since in the strict sense of modern genomic science this means to include all crops and horticultural traits having been modified also by conventional breeding. This kind of now false but routine denomination is a symbol for the disregard of proper science in regulation.

A further comparison demonstrates that legislations in Europe and Brazil are both rather strict, the decisive difference is that in Brazil there are clear (political) decision-making rules, whereas these are lacking in Europe. Until lately, the decisions were depending on majority voting rules of the European states, and this caused a lot of confusion and an almost complete stall in decision-making. This is why Commissioner Dalli [153], in July 2010 opened a debate on delegating some important decisions to the national level: Comments from http://www.gmo-compass.org/eng/news/523.docu.html

- (13 July 2010) As expected, the EU Commission decided on 13.07.2010 changes in the legal regulation of green biotechnology. Accordingly, Member States should be able to prohibit the cultivation of genetically modified (GM) crops that have been approved EU-wide. As the next step, the EU Parliament and Council of Ministers must agree.

The outcome will again depend on complex negotiations and it is not sure whether Commissioner Dalli and the EU will come to concrete legislative results. And, except for some modest GMO corn cultivation in Spain, the present day acreage of GM cultivars remains disappointingly low [154].

In contrast to the complex and stalled situation on European GMOs, the case in Brazil documents in the last few years successful regulation of GMOs: Recent reports document steadily growing acres on GMO crops in Brazil: [155, 156]

- The 1st survey on agribiotechnology in Brazi l for the 2010/11 growing season showed there was a substantial growth in the adoption rate of biotech soybeans, corn, and cot ton. The Brazilian farmers are expected to plant 17.2 million hectares with GM soybean cultivars, or 75.6% of the total harvested surface, in 2010/11.

For a general survey of the Brazilian situation, see the recent publication of Mendonca-Hagler et al. [157], where a clearly optimistic picture is developed. The abstract reads:

- Biotechnology is a Brazilian priority, and has been recognized for its potential to promote sustainable development. The Government recently announced an ambitious program for Science and Technology, which includes strategies to develop modern biotechnology, continuing three decades of public investments on capacity building and infrastructure, aimed principally at the development of technologies applied to health, agriculture and the environment (MCT 2008 http://www.mct.gov.br/). Research initiatives have focused on genomics, proteomics, genetically modified organisms (GMOs), gene therapy, stem cells, bio-fuels and nanotechnology, among other biotechnological topics. Research projects in Brazil have been mainly developed in public universities and institutions funded by federal and state agencies, with a minor participation from the private sector [158]. Genomics, an area of considerable success in the country, was launched a decade ago by S. Paulo State Research Foundation (FAPESP), with the organization of a virtual institute, called ONSA, comprising several laboratories with the main task of sequencing the genome of the citrus pathogenic bacterium Xylella fastidiosa [159, 160].

The success of this genomic network stimulated biotechnology startup companies and projects with the focus on other genomes, such as sugarcane and coffee, including functional genomics and proteomics. Following in the footsteps of the ONSA network, the Ministry of Science and Technology created a National Genome Project Consortium involving institutions located in the major regions of the country, with the task of sequencing eight microbial and two plant genomes. Recently, they concluded the sequence of Chromobacterium violaceum, a bacterium with exploitable properties, such as the ability to produce a bactericidal purple pigment (violacein) and bioplastics [161]. Later on, several states launched their own genome programs. A group from Rio de Janeiro, part of the Riogene network, recently sequenced the genome of the nitrogen-fixing bacterium Gluconoacetobacter diazotrophicus, a sugarcane endophyte involved in enhancing growth of large crops without the addition of nitrogen fertilizer [162, 163], see also the websites of EMBRAPA http://www.embrapa.br/english and the Ministerio Biotecnologia e Tecnologia http://www.mct.gov.br/.

Agriculture plays an important role in the Brazilian economy, being responsible for ca. 40% of the exports and employing 20% of the active work force. About

> one third of the Brazilian GDP comes from agribusiness. Traditionally, this country has been competitive in tropical agriculture, supported by strong research programs on conventional and modern technologies. Intense capacity building initiatives resulted in the formation of a critical mass of scientists working in molecular biology and agricultural sciences [158]. Despite these favorable factors, the adoption of GM crops has been delayed due to intense opposition organized by environmental groups and additional difficulties resulting from a conflicting regulatory framework. In this overview, we address the current status of Brazilian biosafety legislation, and discuss the perspectives for the development of molecular biotechnology in Brazil.

This view is confirmed in a recent editorial in *Nature*, [164], interestingly enough with the same emphasis as above on gene sequencing projects which are the basis of independent biotechnological research and development in Brazil.

Also, the latest success of approving regulatory decisions is symptomatic of the positive biotech climate in Brazil: The first fully developed transgenic crop in Brazil has been approved for commercialization, published in 2007: [165]. The press release of the president of AnBio (National Biosafety Association) Leila Oda emphasizes also the socioeconomic importance of this approval: [166].

Without going into a survey on the Brazilian opponent's activities and reports in detail, here just a typical example published by a medical group (not linked in any way with environmental toxicology) [167] on how science is distorted in order to make a negative and totally unfounded point against glyphosate is given. This paper produces negative toxicological effects on clearly doubtful experimental scenarios: experimental Xenopus frog embryos were *injected* with glyphosate, as mentioned in the introduction.

- We show here that sublethal doses are sufficient to induce reproducible malformations in Xenopus and chicken embryos treated with a 1/5000 dilution of a GBH formulation (equivalent to 430 μM of glyphosate) or in frog embryos **injected with glyphosate alone (between 8 and 12 μM per injected cell).** GBH treated or glyphosate injected frog embryos showed very similar phenotypes, including shortening of the trunk, cephalic reduction, microphthalmy, cyclopia, reduction of the neural crest territory at neurula stages, and craniofacial malformations at tadpole stages.

This absurd experiment methodology contradicts all internationally agreed rules on environmental toxicology testing, as described and cited in detail in [168].

But opponents are well organized on an international level, and promptly, the Paganelli paper is cited in many of those reports, here is just one example: [169]. In this extensive report, dozens of papers are cited which do not match the high quality standards of biosafety science; they are cited because they produce negative results related to modern soybean agriculture. The following is an example on how the authors do not even shy away from distorted reporting of published results.

- Very few studies directly examine the effects of GM foods on humans. However, two studies examining possible impacts of GM RR soy on human health found potential problems.

  Simulated digestion trials show that GM DNA in GM RR soy can survive passage through the small intestine and would therefore be available for uptake by the intestinal bacteria or cells [170]. Another study showed that GM DNA from RR soy had transferred to intestinal bacteria before the experiment began and continued to be biologically active [171]. These studies were not followed up. GM proponents often claim that GM DNA in food is broken down and inactivated in the digestive tract. These studies show that this is false.

Actually, if you read the above Newcastle study properly, you notice that the GM DNA is completely decomposed in the colon, the only traces measurable were found in fresh, undigested stomach probes of human ileostomy patients. Reading the summary alone shows the blatant incorrectness of the comments. Two previous studies, after careful reading, reveal the same results [170, 172]. The conclusion therefore is that the interpretation of [169] is false, as confirmed in the latest publication of the Newcastle research team:

- The transgene did not survive the gastro-intestinal tract of human subjects fed GM soya.

A recently published paper of Zhang is seen as a breakthrough in our knowledge on interkingdom relations between plant and animal genomics: [173].

First data, obtained with modern genomic analysis, demonstrate the surprising finding that exogenous plant miRNAs are present in the sera and tissues of various animals and that these exogenous plant miRNAs are primarily acquired orally, through food intake. MIR168a is abundant in rice and is one of the most highly enriched exogenous plant miRNAs in the sera of Chinese subjects. In addition, these findings demonstrate that exogenous plant miRNAs in food can regulate the expression of specific target genes in mammals.

This could lead to erroneous conclusions that horizontal gene transfer is possible also for the antibiotic resistance genes and even for genes expressing Bt toxins into mammals and humans, and one can see already that opponents to genetic engineering take advantage of the news by clear misinterpretation of the results: They use it as an argument for the unforeseen risks of the technology. See the comments of anonymous scientists in GMwatch [174]:

- The study is yet another nail in the coffin of the already discredited 'safety assessment' process for GM foods in the EU and elsewhere. These assessments do not consider the effects described.

This rather naive statement is typical of the thinking of GM crop opponents: Firstly, they mix up in an unscientific way various categories of transgenes; secondly, they mix up scientific progress and the inevitable adaptation of risk assessment methodology with the present day regulatory rules in place in the laboratories. It is a matter of simple scientific consensus that biosafety assessment has to adapt in methodology with the progress of genetic engineering: on one side, Zink-Finger and TALES methods (details see Zinc Finger Targeted Insertion of Transgenes and TALEs: Transformation Method Transcription Activator-like Family of Type III Effectors.) with all their precision and elegance are prone to simplified risk assessments after detailed studies. On the other hand, technologies using small RNA molecules will undoubtedly force risk assessment researchers to adapt to appropriate methods of analysis, as already proposed by [175]:

- In the future, the predictive ERA process will need to be flexible and adaptable for analysis of the next generation of crops engineered using RNAi and HD-RNAi. As a first step, regulatory agencies and risk analysts need to become familiar with the science of RNAi and its application to plant biotechnology. A concerted effort is needed to develop a pool of expertise to ask the right questions about potential hazards and exposures, to ensure that relevant data are collected and to characterize uncertainty in risk assessments.

  Regulators will have to evaluate the design and implementation of research protocols for laboratory experiments and confined experimental field trials. Scientific questions will need to be answered about off-target effects, non-target effects and the impact of genetic mutations and polymorphisms. Understanding the stability, persistence and half-life of small RNAs in various aquatic and terrestrial ecosystems will be essential for the characterization of exposure pathways. New diagnostic tools will probably be required for the identification and quantification of small RNAs for a range of purposes, including crop identity preservation, monitoring and segregation. Ideally, these tools should have a low detection limit and a high degree of specificity for each RNAi crop, while being relatively inexpensive, functional under field conditions and operable by individuals with diverse backgrounds and training. With all this in mind, it should be possible for stakeholders, regulators and citizens to develop policies and ERA frameworks for RNAi and HD-RNAi crops. [175]

It is correct that small RNA molecules are considered and used for GM plant improvements, as suggested by [175]. And it is also correct that the risk assessment of GM crops up to now does not specifically include the effects described by Zhang et al., that is, that small miRNAs are obviously passing mammal stomach environments and can be integrated in the organism and even be active genetically. This seems to be routine in the evolution of life (and undoubtedly calls for verification and further studies). And the question arises whether we should *automatically* include in the risk assessment small miRNAs, the answer should be *no*: rather it should be another reason to switch European and UN-Risk assessment to product-oriented mood, following the conclusions drawn in the section on the GM- and Non-GM-crop Differences Overestimated, the "Genomic Misconception".

The above examples of misleading statements and publications of the opponents lead in a logical way to the following section on the quality of scientific papers:

Peer Review in the Biosafety Science Debate on Regulation

Before we start talking about regulation, a word on the science debate shall precede, which depends on the process of peer review, but it may be flawed in many ways, although there is no real good alternative in sight, despite some attempts to change this situation like the proposal to involve respected science journalists. But there are objections: journalists might become part of the system [176] and give up indirectly their strict impartiality and neutrality – which is, maybe, anyway an illusion. Or it might be that they may simply not have the scientific expertise as demonstrated recently in a contribution of a science journalist in Nature [177], extensive critical comments in ASK-FORCE contribution on the Rosi-Marshall publication on aquatic insects, see [178] (more comments about this study are given below). It should also be admitted, that a fresh look of a "greenhorn" might reveal new aspects of the GMO battle.

The quality of biotechnological research is also influenced by the research environment offered to students and is evaluated in a differentiated way for Europe by Reiss et al. [179]. Peer review is a very fragile instrument and needs constant inquiry, as demonstrated also on the Wikipedia website on the subject of peer review http://en.wikipedia.org/wiki/Peer_review. It should also be seriously considered that the present day peer review system is basically "faith based," as described with convincing details by [180].

A trend toward a magazine style is documented for some important journals as *Nature* and others. The facts show that the percentage of externally peer-reviewed articles has dropped dramatically. Facts will be given in a forthcoming publication of R. Laporte, F. Linkov, and K. Ammann.

We should also include a new element in the reviews and evaluation of science as proposed by Lubchenco [181]: the scientific community should formulate a new *Social Contract for science.*

> This contract would more adequately address the problems of the coming century than does our current scientific enterprise. The contract should be predicated upon the assumptions that scientists will (1) address the most urgent needs of society, in proportion to their importance; (2) communicate their knowledge and understanding widely in order to inform decisions of individuals and institutions; and (3) exercise good judgment, wisdom, and humility. The paper concentrates, according to the zeitgeist of the publication date, too much on environmental issues alone, today we should put into the center of our science strategy debates **humanity as a whole – and this means to take care of the most urgent needs, namely to work on the eradication of hunger**.

However, this process should not be mollified on the costs of hard science. The line between science and pseudoscience is often difficult to draw.

An interesting new aspect has been introduced by the Supercourse Group with Faina Linkov and Ron LaPorte: [182]. It is true that quality control of Internet texts need rethinking, and it is also important to analyze in a critical way peer review of print material: Their comments can be summarized as follows: High-quality, Internet-distributed lectures are not basically different from written science publications, they also must be documented and references properly. A further element could be a method of quality management introduced originally for the industry by Edwards Deming Wikipedia of Edward Deming http://en.wikipedia.org/wiki/W._Edwards_Deming, who very successfully taught management and quality control also in Japan in the 1950s.

Two more initiatives should be mentioned here, they can be summarized under a kind of *post-publication peer review.*

Faculty of 1,000 System With a total of nearly 84,000 articles reviewed by May 2011, the system has accumulated an important body of comments, see http://f1000.com/, the comments, although really critical sentences are not foreseen, the system is now linked to The Scientist and provides helpful orientation about important publications. Some examples have been evaluated by the author [183].

Frontiers of Science Frontiers of Science has been developed over 2 years in consultation with scientists and other faculty, as well as with students and postdoctoral fellows, to address manifest intellectual, logistical, and pedagogical issues, see http://www.sciencecore.columbia.edu/s2.html and http://www.fos-online.org/

Declaration of a New Global Business Ethos as a Barrier Against Undue Influence on the Publication Policy of Scientific Journals On October 6, 2009, Hans Küng, Josef Wieland and Klaus Leisinger presented the Declaration of a NEW GLOBAL BUSINESS ETHOS at the United Nations in New York http://www.novartisstiftung.org/platform/content/element/3177/Newsletter_3-09_2.pdf.

Although coming from a pharmaceutical company like Novartis, multinational seed companies will (or should) most likely join. Such efforts are important, because there is a constant pressure of undue influence on scientific papers, although resisted successfully by most researchers, but the influence of multinational (in this case pharmaceutical) companies can be hidden but nevertheless powerful:

An example of such influence by units sponsoring scientific journals has popped up in Australia: See the debate around the withdrawal of six Australia-based Elsevier "fake" journals sponsored by the pharmaceutical industry, see the statement of Elsevier's CEO Michael Hansen [184] and [185–187]. This kind of influence might still be under control, and peer review is usually functioning in an unbiased way – but the difficulties are deep-rooted, and it is a constant fight for quality, as is summarized comprehensively by Scott [188].

It is a cheap and intellectually intolerable slogan of opponents of genetic engineering in agriculture when they discredit researchers for their relationships with industry, since the great majority of researchers all over the world act as independent persons, although sometimes also funded by industry. The sole quality criteria on science are transparency in applied methods agreed upon by the science community and the reproducibility of the data. For more details see section More on the Quality of Scientific Publications.

In the "dangerous" waters of corporate influence, we need renewed efforts of scientometric analysis, as given earlier in a report of bio-era: [189]. The top part of table 5 reveals the few really successful seed companies in relation to the top universities with agricultural research regarding R&D (Fig. 9):

The top 35 R&D orgnizations in agricultural biotechnology

RANK	PARENT ORGANIZATION	SHARE OF INDUSTRY R&D OUTPUT
1.	Monsanto	29.82%
2.	Du Pont / Pioneer	10.98%
3.	Bayer / Aventis	10.14%
4.	Dow	5.81%
5.	Syngenta	5.80%
6.	Savia / Seminis	2.57%
7.	USDA	2.38%
8.	BASF	1.71%
9.	Cornell University	1.25%
10.	Stine Seed Farm Inc	1.15%
11.	Florigene	1.08%
12.	University of California	1.05%
13.	Exelixis	0.98%
14.	Iowa State University	0.91%
15.	Rutgers University	0.83%
16.	University of Guelph	0.79%

GM Crop Risk Debate, Science and Socioeconomics. Figure 9

Table 5, upper part, with a ranking of biotech companies and universities in the USA, from [189], calculation rules above

The calculation rules for the table below:

- The four R&D measures are weighted equally. For example, having 10% of industry patents is just as significant as having 10% of commercialized products. Share of industry R&D output = (share of industry patents + share of industry patent citations + share of industry field trials + share of industry commercialized products)/4 [189].

More on the Quality of Scientific Publications Coming back to the peer review on the quality of scientific papers, all the above statements do not mean to say goodbye to the factual and methodological scrutiny per se – even after a paper is already published. With a focus on the GM food safety research Chassy and Parrott [168] summarize the criterions on how to

judge whether a food study is believable or not: (a) Making sure the samples tested are comparable samples. (b) Testing composition to make sure the tests and controls are comparable. (c) The need for an acceptable balanced and nutritious diet. (d) Why the dose is important. (e) What statistics do and do not tell us. (f) The importance of peer review and scientific publication. (g) Guidelines for dealing with conflicting information. (h) Ethical considerations. A very important additional point is emphasized by Kostoff [190]: "Multiple technical experts should average out individual bias and subjectivity." Two blatant examples of lack of peer review properly done are, among others, discussed in ASK-FORCE (with some additions related to recent publications, all cited in the renewed blog:

- The case of Bt endotoxins supposedly affecting aquatic organisms by Rosi-Marshall et al. [191]

  See comments in ASK-FORCE blog No. 3 on Rosi-Marshall et al. 2007b: [178] (including also the latest publications of [192]. The study has been criticized heavily by [193] and [194], the main points of critique, summarized in a letter to the editor of PNAS [195]: No indication about the nature of Bt toxin, nor any data about its origin. Unscientific extrapolation from lab to field experiments, suppression of an important result of Fig. 3: low toxicity of normal Bt toxin levels for aquatic organisms etc. It is good to know that the authors of the original study admitted some mistakes and tuned down their alarmist interpretation in the first study:
- The case of the Austrian mice experiments supposedly affecting fertility after some generations [196]. After lots of public and scientific debate, which caused serious and unfounded damage to the image of Bt crops, the study results were distributed on hundreds of websites of GM crop opponents. But critique came up, and since there was no publication in a peer reviewed journal available, the rebuttals were not published in journals either. The whole bitter debate is summarized extensively in two ASK-FORCE blogs: [197].

  The subsequent official retraction done by the Austrian Government itself is hidden in an European Commission Health and Consumers Directorate-General Summary Record of the Standing Committee on the Food Chain and Animal Health from October 19, 2008: European Commission Health and Consumer Directorate-General, Summary Record of the Standing Committee on the Food Chain and Animal Health Held in Brussels October 19, 2008: http://ec.europa.eu/food/committees/regulatory/scfcah/modif_genet/sum_19102009_en.pdf

  See also the published comments of Ammann in [198]:

> Studies that look at non-obvious risks are a welcome addition to the literature, say critics, but poorly conducted studies do more harm than good. "It's just bad science," says Ammann. "There are a lot of scientists producing these studies in a very sloppy way. They bolster public fear yet do nothing to resolve conflicts or move the field forward". And:
>
> But the authors aren't to blame, says Klaus Ammann, emeritus professor at the University of Bern in Switzerland. They are merely the latest victims of what has become the political gerrymandering of science to bolster and support anti-GM sentiment in Europe. "The Austrian government had exhausted all legal avenues to ban cultivation of GM crops," Ammann says. "The Ministry of Health decided to avoid the peer-review process and announce study results at a conference, hide the data from scientists, and let the activists run amok with the help of uncritical media." Indeed, in the ensuing months the Austrian government has backpedaled. The Ministry of Health responded to a request to interview Zentek or other authors with the following: "We asked the scientists to reevaluate their statistical analysis. Additionally the external evaluation will soon be started. I kindly ask you to wait with your proposal until the reevaluation is completed." [198]

- The case of a review by Dona and Arvanitoyannis [199]. This review would never pass tests designed by Tang et al. [200], which can detect biased filtering of citations and words: According to Tang et al., it is important to distinguish between *subjectivity classification* retrieved from opinionated and factual statements, and combine it with a multiclass *sentiment classification*, and to get a better scale by using neutral training examples. An extensive scientific analysis on [199] has been placed in ASK-FORCE with critical comments: [201]

A caveat at the end of this paragraph on peer review is appropriate. Although it is in principle necessary to ask ethical questions, we should first concentrate on the scientific assessment of a professional peer review strictly following a factual agenda such as [168, 202] are demanding. Only then when this filter has been passed successfully, it is important to go into ethical and socioeconomic questions. But as often, it is the farmers and the market regulating efficiently, and – no surprise – they follow quite naturally socioeconomic principles. It is wrong to mix scientific and ethical questions as de Melo et al. and Interman et al. are asking for [203, 204], the result is then to accept for discussion a paper like the one of [205], which has been seriously and repetitiously criticized on a factual basis by EFSA [206–208]. Such papers should not be seen as a publication which takes also into account a "balanced view," because they are flawed in the first place. Papers from the laboratory of Séralini are then often cited as done by *independent* scientists, which is not very convincing, since digging into the financial support of Séralini and his CRIIGEN lab it is highly interesting to realize that they also receive funds which come from opponents of GMO technology, such as Sevene Pharma, commercializing homeopathic products which claim to detoxify various toxic products [209] and more. CRIIGEN has been created with the financial support of the retailer Carrefour, which has also contributed financially to certain studies of Séralini and his group. Interestingly enough, Carrefour, the second largest food distributor in the world, sells its own brand of "GMO-free" products. . .Source: [210].

- The result of this discussion: it will be necessary to call for new, Internet-based methods to create a more efficient peer review system. A nucleus of such a system is given in Ron LaPorte's supercourse system http://www.pitt.edu/□super1/.

GM- and Non-GM Crop Differences Overestimated, the "Genomic Misconception"

Early Phase of Risk Assessment In the wake of molecular breeding, in particular with the first successes of "gene splicing," the safety debates started soon after the discovery of the DNA structure by Watson & Crick [211–213], followed by the Asilomar Conference [214, 215] – see also some historical accounts [7, 216, 217]. The fascination about the novelty of transgenesis was justified, but also overwhelming, and the many unforeseen scientific breakthroughs following were unprecedented in the history of molecular biology. Unfortunately, the enthusiasm also lashed back in an overacting in risk assessment, when the first GM crops went into production. The debate on how GM crops should be regulated started very early with an emerging divide between regulation in the USA and Great Britain, including later the whole of Europe [218, 219]. Some more traces of early disputes about regulatory decisions in the USA and in Great Britain can be seen in letters to Nature in 1992: [220, 221]. Some support tighter regulation including field biosafety assessments, others fear strangulation of biotechnology research. During the wake of the Cartagena Biosafety Protocol most countries adopted (around 2003) the European way of risk analysis of genetic engineering, emphasizing process-oriented regulation and rejecting product-oriented regulation.

The seemingly absolute novelty of genetic engineering on the molecular level has been contested already in the early days of molecular biology in the 1930s and 1950s with the discovery of cellular systems for genome restructuring discovered with the classic papers of McClintock [222, 223] and with later commentaries of Fedoroff [224, 225], also summarized under "natural genetic engineering" [226, 227].

Molecular Processes Similar in Natural Mutation and Transgenesis Genetic engineering has been brought into evolutionary perspective of natural mutation by authorities such as Werner Arber: his view remains scientifically uncontested that molecular processes in transgenesis and natural mutation are basically similar [228–232]. In a recent paper, Werner Arber [19] reemphasized those similarities on a broader organismal and evolutionary basis; the abstract reads:

- By comparing strategies of genetic alterations introduced in genetic engineering with spontaneously occurring genetic variation, we have come to conclude that both processes depend on several distinct and specific molecular mechanisms. These mechanisms can

be attributed, with regard to their evolutionary impact, to three different strategies of genetic variation. These are local nucleotide sequence changes, intragenomic rearrangement of DNA segments and the acquisition of a foreign DNA segment by horizontal gene transfer. Both the strategies followed in genetic engineering and the amounts of DNA sequences thereby involved are identical to, or at least very comparable with, those involved in natural genetic variation.

Therefore, conjectural risks of genetic engineering must be of the same order as those for natural biological evolution and for conventional breeding methods. These risks are known to be quite low. There is no scientific reason to assume special long-term risks for GM crops.

For future agricultural developments, a road map is designed that can be expected to lead, by a combination of genetic engineering and conventional plant breeding, to crops that can insure food security and eliminate malnutrition and hunger for the entire human population on our planet. Public-private partnerships should be formed with the mission to reach the set goals in the coming decades. "from [19].

The same claim is made with a more organismic view by Hackett [233].

It is therefore no surprise that a natural transgene species has been discovered in a widespread grass genus [234]. An extensive overview on "natural transgenic organisms" is given in the excellent blog of David Tribe GMO pundit on natural transgenics: http://gmopundit2.blogspot.com/2005/12/collected-links-to-scientific.html.

Recent publications demonstrate that transgenesis, for example, has less impact on the transcriptome of the wheat grain than traditional breeding [235–237] (more details see [44, 238]).

One should also take into account that many of the conventional breeding methods such as colchicination [239, 240] and radiation mutation breeding [241] can be obviously more damaging to the genome, and it is, in addition, not possible to clearly define what impact the untargeted process could have caused. Or, on the other hand, as [242] have demonstrated, that irradiation-induced wheat – Aegilops biuncialis intergenomic translocations will facilitate the successful introgression of drought tolerance and other alien traits into bread wheat. In their review, [243] criticized the biased statements of [244, 245] who focus in an unjustified manner on transgenesis alone when describing unwelcome mutations. Still, it has to be admitted that repair mechanisms on the DNA level are powerful [246–248]. It is thus not logical that opposition within organic farming toward genetic engineering is now expanding also to some of those conventional breeding methods, some go even so far as to reject marker-assisted breeding – symptomatic for the organic agriculture scene, this trend is based on the myth of "intrinsic integrity of the genome" [249, 250], for which term it is not possible to find a proper scientific definition, which inevitably should be based on comparisons [44]. The addition of rejected breeding methods would ultimately lead to an absurd situation where most of the modern time traits would have to be rejected and breeding would be forced to virtually start from scratch.

Basically, many of the first-generation GM crops should be today subject to a professional debate on *deregulation*, and there is good and sturdy reason to state that many of these GM crops should not have been treated in such a special way in the first place, they can be compared in their risk potential to many crops created with traditional methods.

- This should not be misunderstood as a plea for general deregulation of GM crops, rather for a strictly science-based, risk-based regulation and clearly for a shift from process-based regulation toward product-based regulation.

Dissent over Differences Between GM- and non-GM Crops Causes Transatlantic Regulatory Divide This actually includes a critical questioning about some basic rules of the United Nations Convention on Biological Diversity (CBD). Transgenic crops of the first-generation should not have been *generally* subjected to regulation purely based on the *process* of transgenesis alone; rather it would have been wiser to have a close look at the *products* in each case, as John Maddox already proposed in 1992 in an editorial in Nature [251]. This is also the view of Canadian regulators [252–254], where the *novelty* of the crop is the primary trigger for regulation. This transatlantic contrast has been

commented by many [16, 218, 255–258], and although for many years a solution and mediation seemed to be too difficult, contrasts can be overcome:

In a letter to the executives of the Convention on Biological Diversity (CBD), the Public Research and Regulation Initiative (PRRI) http://www.pubresreg.org/index.php?option=com_docman&task=doc_download&gid=490 is asking for a scientific discussion in order to exempt a list of GM crops from the expensive regulatory process for approval, here is only the final statement:

- Bearing in mind that the **method of transformation itself is neutral**, *i.e.,* that there are no risks related to process of transformation, PRRI believes that there are several types of LMOs and traits for which - on the basis of the characteristics of the host plant, the functioning of the inserted genes and experience with the resulting GMO - **it can be concluded that they are as safe as its conventional counterpart with respect to potential effects on the environment, taking also into account human health**.

Unfortunately, there was no substantial reaction from the leading Cartagena organizers.

To be quite explicit once more, this does not mean to exempt transgenesis from biosafety assessment as a whole, but it should say that "several types of LMOs and traits, where the inserted genes demonstrate in large scale commercialization (of course after risk assessment done in due course) can be deemed as safe as conventional counterparts according to several years of beneficial agricultural practice, should be exempt under article 7.4 of the Cartagena Protocol for further expensive and time-consuming risk assessment and regulatory procedures. This motion has now officially been repeated by PRRI (Public Research and Regulation Initiative at the occasion of the COP10-MOP5 negotiations in Nagoya, Japan, see the interventions on the website www.pubresreg.org with recent additions.

In a recent paper, an indiscriminate continuation of food biosafety research is questioned on the basis of all the above arguments by Herman et al. [259] with good reason:

- Compositional studies comparing transgenic crops with non-transgenic crops are almost universally required by governmental regulatory bodies to support the safety assessment of new transgenic crops. Here we discuss the assumptions that led to this requirement and lay out **the theoretical and empirical evidence suggesting that such studies are no more necessary for evaluating the safety of transgenic crops than they are for traditionally bred crops.**

Perspectives for Solutions, a Synthesis of Divergent Views in 2.4

These new perspectives create hope that solutions can be found. Even within the difficult and for GMOs totally negative legal environment of the Cartagena Protocol, there are some slim possibilities:

In a first phase some of the widespread transgenic crops like transgenic maize with the Cry1Ab endotoxin could be exempt from regulation. This is indeed possible according to art. 7.4 in the Cartagena Protocol. In COP-MOP5 2010, in Japan (Fifth meeting of the Conference of the Parties serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety (COP-MOP 5), 11–15. 10. 2010 Nagoya, Japan http://bch.cbd.int/protocol/meetings/) it should be possible, to amend the protocol with the introduction of a dynamics which allows to start the regulatory process with an initial phase focusing on the process of transgenesis, first following procedures proposed for nontarget insects by [260, 261].

Indeed, in COP10-MOP5 in Nagoya October 2010, PRRI www.pubresreg.org has made a request for the exemption of widely adopted Bt maize crops of the endotoxin type of Cry1Ab, see the press release for the context (PRRI press release: http://www.pubresreg.org/index.php?option=com_docman&task=doc_download&gid=586), here the original text as read at the plenary meeting in Nagoya: PRRI Statement on exemptions MOP5: http://www.ask-force.org/web//PRRI-MOP5/PRRI-MOP5-statement-Strategic-Plan-delivered.pdf:

- Third, there is an underlying misperception that there are demonstrated cases of adverse effects. This is incorrect. Over the last 15 years GM crops have been planted over a billion hectares by tens of millions of farmers in the developing and developed world. These crops

have been grown in numerous different environments, and they have been consumed in billions of meals. The substantial scientific evidence accumulated shows that there are **no** verifiable reports of any adverse effect to environment or human health.

The Strategic plan includes an indicator "Number of reports to the BCH on the identification of LMOs or specific traits that may have adverse effects". Such an indicator makes little sense, because it is never possible to rule out that any organisms, LMO or non LMO, may have adverse effects. What is crucial is the question whether they are likely or unlikely to have adverse effects, and PRRI proposes that the strategic plan includes these two questions. PRRI is ready to submit examples of categories of LMOs of which the risk assessments and accumulated evidence indicate that they are unlikely to have more adverse effects on biodiversity or human health than their non modified counterparts, and that consequently those LMOs can be exempted from the AIA procedure on basis of article 7.4 of the Protocol.

In future, it should also be possible to shift eventually the focus on the product, making it possible to abbreviate the regulatory process wherever possible and feasible. The ultimate goal of new regulatory concepts should be to minimize obstacles for new and urgent necessities in crop development, such as Swaminathan and Raven are proposing [262, 263]. The author remains pessimistic, since the whole cumbersome process of legal changes in the Cartagena Protocol is also systematically hindered by a strong anti-GMO lobby, having made its way through the institutions to higher and powerful positions within the Cartagena administration quite successfully, starting from MOP1 all the way up through MOP5, thus influencing negatively all change of regulatory appeasement and lowering regulatory costs. Unfortunately, the recent overview of the European legislation on GM crops does not generate much optimism either: [264].

A second negative trend is triggered by a growing community of risk assessment researchers, who have a vested interest to keep the pot cooking, examples can be downloaded at the website of GENOK www.genok.com and also from the website of the Third World Network http://www.twnside.org.sg/ with its intricate mixture of activist statements and questionable and peer-reviewed scientific contributions. Other similar examples supporting this view can be downloaded over the Freiburger Oekoinstitut http://www.oeko.de/ and on the website of ENSSER, European Network of Scientists for Social and Environmental Responsibility http://www.ensser.org/

A conceptual framework is proposed by IFPRI/ISNAR in 2002, the International Service for National Agricultural Research [265]; a careful evaluation of process-based versus product-based triggers in regulatory action can also lead to a merger of both seemingly so contrasting concepts into a legalized decision-making process on which trigger should be chosen in a case-by-case strategy:

- Process-based triggers are the rule in almost all countries that have developed national biosafety regulatory systems; there are exceptions, however, where the novelty of the trait determines the extent of regulatory oversight and not the process by which the trait was introduced. While such a product-based approach to defining the object of regulation is truest to the scientific principle that biotechnology is not inherently more risky than other technologies that have a long and accepted history of application in agriculture and food production, it is less prescriptive than process-based regulatory systems.

Many of the debates on those two concepts suffer from a lack of clear-cut definitions, it will be important to have a close look at the Canadian regulatory system and the definition of PNTs (Plants with Novel Traits). In Canada, the trigger for risk assessment is the *novelty* of the plant rather than the *methods* used to produce it. The difficulties start there, where a clear definition of PNTs is needed to come to a decision. It means that plants produced using recombinant DNA techniques, chemical mutagenesis, cell fusion, cisgenics, or any other in vitro technique leading to a novel trait need to undergo risk assessment in the Canadian system. No wonder the Canadian definition of novel traits is rather wordy, but remains broad minded:

- A plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated seed in Canada and that have

been intentionally selected, created or introduced into a population of that species through a specific genetic change.

Conclusions: There can be no doubt that product-based regulatory approaches are truest to the scientific principle that biotechnology is not inherently more risky than other technologies that have a long and accepted history of application in agriculture and food production, it is also less prescriptive than process-based systems, see for more details McLean et al. [265].

The Costs and Lost Benefits of Overregulation

The Issue

The Cartagena Protocol on Biosafety (CPB) has now been adopted by 157 parties http://www.cbd.int/biosafety/signinglist.shtml. It still builds on the principle that GM crop plants might bare risks in contrast to the conventional crops, objective of CPB: http://www.cbd.int/biosafety/articles.shtml?a=cpb-01. The huge apparatus on risk assessment based on this protocol is building on the principle that the mechanism of transgenicity is totally artificial and is not found in nature. Modern molecular science insights have proven the contrary, as shown in ASK-FORCE AF-9 [201] on the molecular basis of transgenesis. This results in maintaining the concept of an asymmetric risk assessment of innovation of GM crops. The possible exemption of widespread GM crops in Art. 7.4 (Cartagena Protocol on Biosafety, Article 7: http://www.cbd.int/biosafety/articles.shtml?a=cpb-07) is not even considered officially up to now.

Summary

An excellent summary graph is given in [266] in Fig. 10b: innovations active in the R&D pipeline were growing at an increasing rate during the period before 1998, but declined after 1998. Apart from competition of reasonably close nontransgenic substitutes, the authors consider one regulatory reason to be the main culprit: The halting of regulatory approvals in 1998 in Europe. Although the authors consider the full extent of reasons still to be conjectural, their data suggest that changes in regulatory environment may have been a cause. In a combination of high costs for lost implementation and high costs for regulatory approvals, the present state and operational experience has grown into a major obstacle of modern crop breeding (Fig. 11).

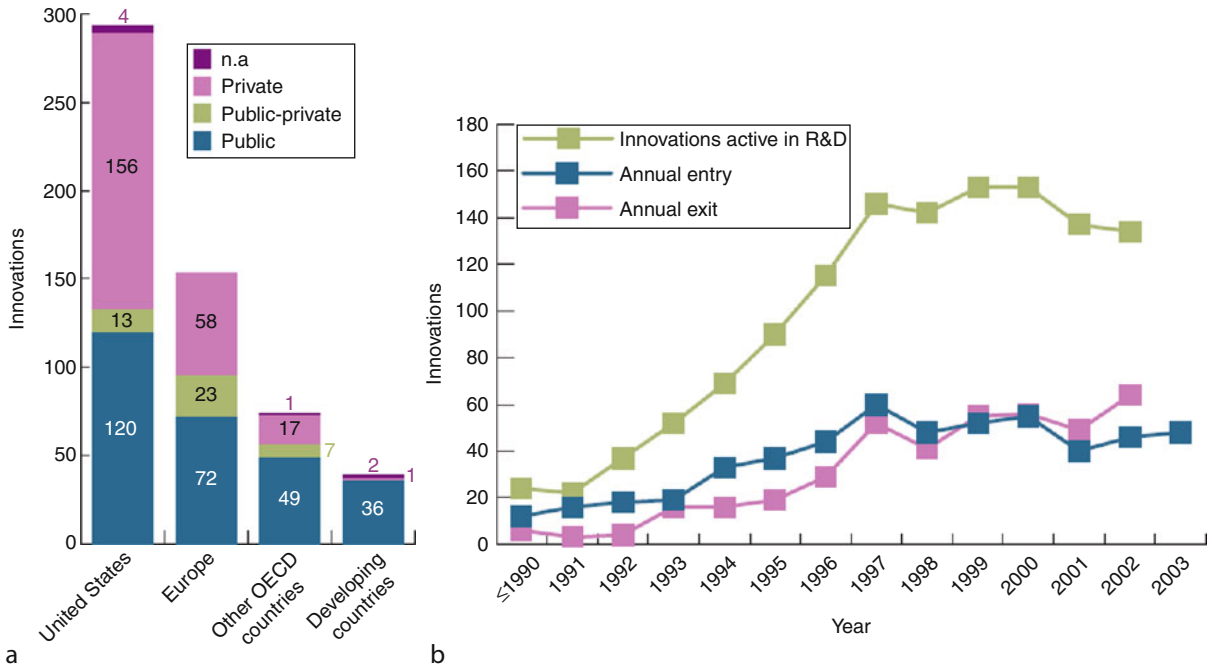
- Commentary from Table 1 in [266]: The primary survey combined records from scientific publications, field trial records, and regulatory filings to identify 558 transgenic plants with quality improvements and determine how far they had progressed through stages of R&D by 2004, including those that had only been published in the scientific literature; those that had reached initial field trials (defined as having completed 1–3 field trials), mid-stage field trials (4–9 field trials) or advanced field trials (>10); those that had entered regulatory filings; and those that were commercialized. The secondary survey canvassed expectations of firms and analysts about the likelihood and time frame for future commercialization of transgenic product quality innovations. Complete one-to-one correspondence between individual observations of the two surveys was not possible.

In a recent publication [267] document the same dramatic negative trend for specialty GM crops is demonstrated:

Costs and Lost Benefits Worldwide and Europe

An excellent summary graph is given in [266] in Fig. 6 above: innovations active in the R&D pipeline were growing at an increasing rate during the period before 1998, but declined after 1998. Apart from competition of reasonably close nontransgenic substitutes, the authors consider one regulatory reason to be the main culprit: The halting of regulatory approvals in 1998 in Europe. Although the authors consider the full extent of reasons still to be conjectural, their data suggest that changes in regulatory environment may have been a cause.

The full extent of the GM crop development pipeline can be evaluated in websites like the Information Systems for Biotechnology alone from the USA, there are (October 23, 2009) 14,204 notifications with 1,586 full field release permits registered in this Database, ISB: Information Systems of Biotechnology: Field Test Releases in the US: http://www.isb.vt.edu/cfdocs/fieldtests1.cfm



GM Crop Risk Debate, Science and Socioeconomics. Figure 10

Innovation in Ag-Biotech. (**a**) Location and sector of organizations conducting R&D for the 558 transgenic product quality innovations identified. Private sector consists of corporate and privately held firms. Public sector consists of government research laboratories, universities, and nonprofit research institutes. (**b**) Annual entry, exit, and the numbers of innovations active in the R&D pipeline were calculated from observations of the 558 innovations tracked in the primary survey. The number of active innovations stopped growing in 1998, after which those new innovations that entered were more likely to be published and less likely to move toward commercialization. Figure 1 from [266]

Overall, the present day regulatory regime detains public research in molecular breeding considerably due to enormously high regulation costs. More information about this effect on the development of GM trees is in Strauss and McLean [268, 269]; the abstract reads:

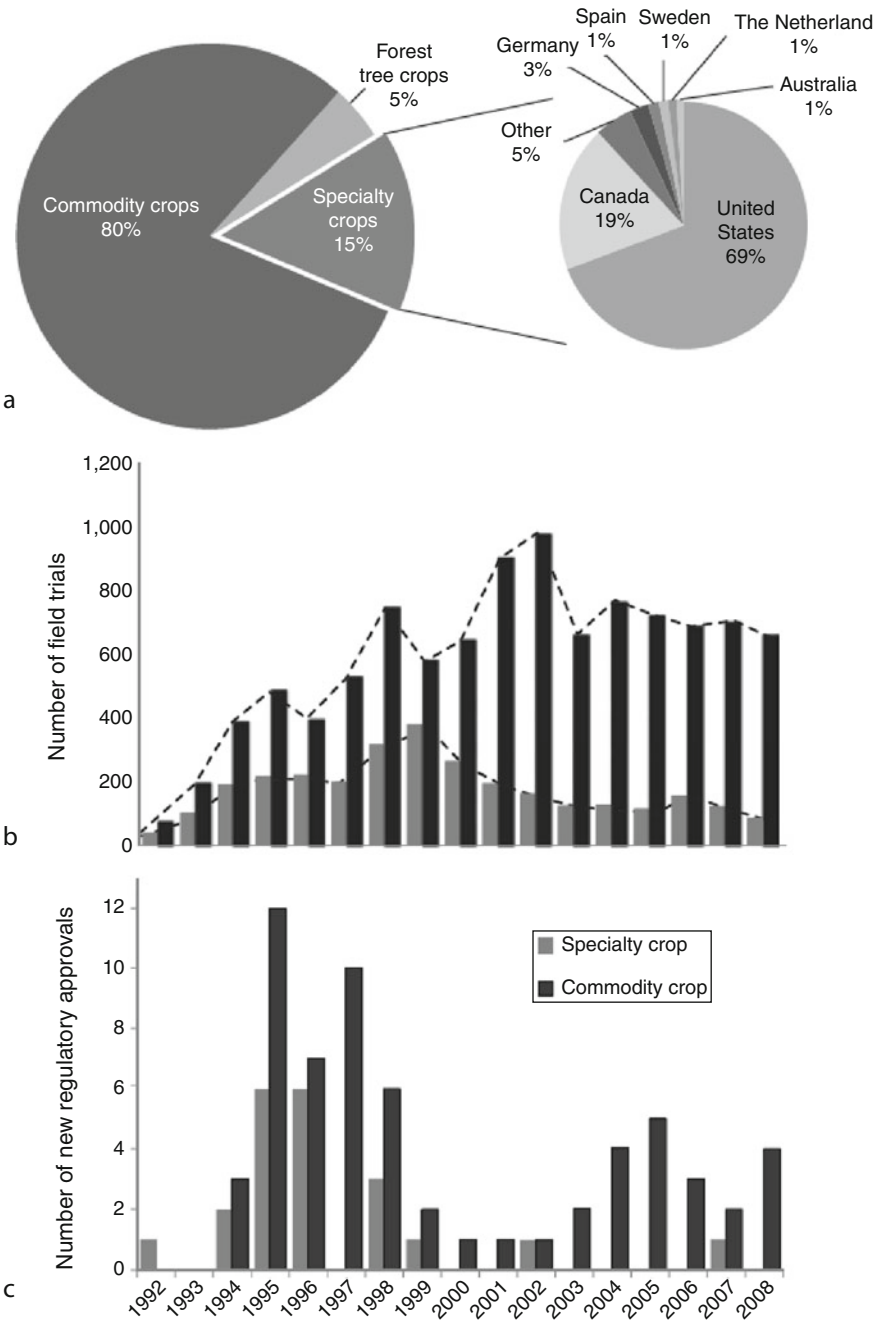
- **Against** the Cartagena Protocol and widespread scientific support for a case-by-case approach to regulation, the Convention on Biological Diversity has become a platform for imposing broad restrictions on research and development of all types of transgenic trees.

Some comprehensive tables on the massive costs of regulation of the major commodity crops are given by Kalaitzandonakes [270]. The compliance costs for herbicide tolerant maize alone have been calculated based on the events in 2006 for the USA. They amount to US $6,180,000–14,510,000 – a sum most likely to be prohibitive for any trait developed by a public institution.

Another case is reported by Piero Morandini from Italy. A scientific assessment on a field trial on Bt maize is delayed in publication by the Italian Government, although (or because?) it yields very positive results [271, 272].

- The grain yield data (tons/ha, GM crop *vs*. their conventional counterparts) were rather spectacular: 15.9 *vs*. 11.1 and 14.1 *vs*. 11.0, translating into a 43 and 28% yield increases for the P67 and Elgina, respectively. These data have already been released by the INRAN (National Institute for Research on Food and Nutrition, a research institution funded and run by the government) in 2006, albeit without the emphasis they deserved.

  The delay in properly communicating these data can be considered as a very costly omission. In fact, taking into account the total area of maize cultivation in Italy together with yield differences, maize prices



GM Crop Risk Debate, Science and Socioeconomics. Figure 11

Field trials and regulatory approvals. (**a**) Using the UNU-MERIT database, field trials conducted in 24 developed countries between 2003 and 2008 were separated on the basis of commodity, forest tree, or specialty crop. From this, the specialty crops were further subdivided based on the country in which the field trial was conducted. (**b**) The numbers of field trial permits acknowledged or issued in the USA are plotted by year for commodity crops and specialty crops. (**c**) The 84 unique transgenic events that have been granted regulatory approval by one or more countries are plotted by year of approval. If the year of approval varied among countries, the first year of regulatory approval granted by any agency for a given event was used. From [267]

and pest pressure, **these data translate into a forfeited value of between roughly € 300 million and € 1 billion a year because Italian farmers are not allowed to plant Bt maize.**

A summary of the Lombardia maize case has also been published in Nature Biotechnology [273]. Unfortunately, the original research report is still not published, it is "resting" in an Italian government drawer. . .

The present day regulatory "cropping apartheid" of high tech farming versus organic farming, large-scale farming against smallholders seriously hampers the development of GM crops, which could foster a more ecological production [44, 274] [275] and [276] – in short, Gene Peace instead of Greenpeace.

Costs and Lost Benefits in Developing Countries

Even more drastically, in the developing world, there is regulatory legislation in place hindering the development of transgenic crop breeding for the benefit of the poor, Driessen, Herring, Paarlberg [277–280].

Doubling agricultural research investment per se (no regulatory costs included in the calculation), would reduce poverty in Sub-Saharan Africa by 9% according to Alene & Coulibaly [281]. But these prospects are seriously hindered and as a result are practically nullified by the exorbitantly high regulatory costs during the implementation phase. Moreover, GM-free private standards set up by food companies and distributors in developed countries have influenced biosafety policymaking in developing countries: Gruère and Sengupta [282] found 29 cases where private importers have affected policy decisions in numerous countries due to irrational fear of export losses. This is based on two generally misleading premises: (1) Europe or Japan represents the only market for exports, and (2) non-GM segregation is too costly. It is amazing to realize, that many of the cases rely on unpublicized lobbying activities, and because of the lack of comprehensive evidence, many cases do not provide straightforward evidence of causality links between importers or traders and policy decisions. There is evidence that development of GM crops in Africa is mainly based on public research, and that the private sector only reluctantly invests in projects for developing countries, although the situation is getting better in the last few years [283, 284].

A blatant case of eco-imperialism is reported from Zambia by Andrew Apel in GMobelus: http://www.gmobelus.com/news.php?viewStory=234, where the Norwegian Government has partly sponsored a $400,000 laboratory, for which GENOK has contributed equipment and training, thus guaranteeing a research policy hostile to GM crops, in accordance with the official policy of the Zambian government, that characterizes GM crops as poisonous. The Norwegian GENOK is a well known anti-biotech NGO, with a very negative attitude toward GM crops, not shying away from spreading myths on allergy caused by pollen of transgenic maize in the Philippines; This is documented in the controversy between GENOK and Rick Roush: http://www.botanischergarten.ch/Allergy/Traavik-Roush-Philippines-controversy-2004.pdf, also supported in favor of Genok without a shred of evidence by John Vidal from the Guardian: http://www.guardian.co.uk/science/2004/feb/27/gm.science. Typically enough, the laboratory's priority will be to detect and search for genetically modified seeds and crops. Former Zambian researcher Ed. Rybicki, now working in Cape Town, said "that the lab would better serve Zambia and the whole region by looking at genuine threats, studying local biodiversity and even making transgenic crops themselves", as reported by SciDev Net http://www.scidev.net/en/news/zambia-s-molecular-biology-lab-fully-functioning-a.html?utm_source=link&utm_medium=rss&utm_campaign=en_news. Indeed, it is rather ironical that many of the biosafety educational efforts undertaken by organizations, highly critical to transgenesis, are turned into the "contrary": the biotechnological methods introduced in those countries are now also used for research and development of GM crops. A comprehensive report on agricultural biotechnology by Alhassan [285] demonstrates that high regulatory hurdles would hinder a reasonable development of modern agriculture in Africa.

Gruère and Smale [286, 287] report in a carefully calculated assessment that if rice cultures in India, Bangladesh, Indonesia, and the Philippines would be based on present day GM traits, the benefits amount to US$4,331 million. For the USA, an earlier assessment calculates similar sums of benefits related to the introduction of biotechnology in agriculture [288].

There has been much more written about regulatory costs and their negative follow-ups. Here only a small selection of important papers [130, 261, 289–294] is given.

The Golden Rice Development Hampered Through Overregulation. Biofortification as an Ideal Sustainable Way of Foreign Aid in Agriculture

In the case of the Golden Rice this tedious and costly regulation forced upon the regulatory authorities by the CBD solely based on the process of transgenesis has serious ethical consequences as documented in http://www.agbioworld.org/biotech-info/topics/goldenrice/index.html and in [270, 295]. A delay of the introduction of the biofortified rice is directly causing each year hundreds of thousands of children to die or to go blind due to severe vitamin A deficiency. Unreasonable and unscientific regulatory obstacles cause massive delay in approvals, especially in developing countries of S.E. Asia [296–311]. The initiator of the Golden Rice Ingo Potrykus project complains bitterly about the unjustified delays due to overregulation in a Nature article: [312].

Specifically related to the developing world, we should refrain from the old myths that international corporate companies are dominating the field – on the contrary Public Research is responsible for 85% of crop developments, 7% private local companies, and only 1% multinational companies according to figures from Cohen [284], supported by FAO statistics [313]. The myth that patenting rules are seriously hampering the spread of helpful biotech crops in poor countries has been seriously contested [314–316].

As an example, the Golden Rice project will result in biofortified rice traits, which will be distributed to the farmers free of royalties. The Asian farmers will also be able to multiply seeds without paying royalties. The homepage of the project is the main information source http://goldenrice.org/. More about the subject can be found in the important and comprehensive Handbook of Intellectual Property Rights of Krattiger et al. 2007 [317], and more: [318–321].

Biofortification programs are prone to get the highest index numbers in the evaluation system for foreign aid programs of Lempert [322]. Biofortification of indigenous landraces by systematically crossing-in the valuable and royalty free traits to enhance the nutritional value is certainly one of the best ways to sustainably help indigenous people suffering from any kind of malnutrition. In all cases known, the technology transfer is royalty free, secured by contracts.

Use of an indicator to assess the quality and success of developing aid projects defined by [322] reveals that most of the major NGO and UN actors in the field of development are actually providing relief rather than development and are creating dependency by treating symptoms rather than long-term solutions. The indicator points to the specific areas where they need to improve in order to fulfill sustainability criteria including tests of whether aid distorts financial markets and business competition, erodes appropriate government functions, and reverses colonial institutions and ideologies that interfere with sustainable consumption within a resource base.

Estimates in costs for vitamin A capsules are clearly incompatible with the living standard in developing countries; a major distribution campaign would result in millions of dollars. Neidecker-Gonzales [323] produced in their study the following figures:

- Total costs are lowest (roughly US$0.50 per capsule) in Africa, where wages and incomes are lowest, US$1 in developing countries in Asia, and US$1.50 in Latin America. Overall, this study derives a much higher global estimate of costs of around US$1 per capsule.

A bibliography of publications of the Golden Rice and Biofortification demonstrates the importance of this field of research; out of a general bibliography of 1,640 references a list of over 200 important papers is assembled: http://www.botanischergarten.ch/Golden-Rice/Bibliography-Golden-Rice-WOS-KA-20091008-links-abstracts.pdf.

It should be mentioned that biofortification strategies are also proposed for feed [324]. Straw from harvested crops can be adapted to higher feeding straw quality for cattle.

Conclusions drawn by Ingo Potrykus [325], the creator of the Golden Rice:

- The huge potential of plant biotechnology to produce more, and more nutritive, food for the poor will be lost, if GMO-regulation is not changed from

being driven by "extreme precaution" principles to being driven by "science-based" principles.

Changing societal attitudes, including the regulatory processes involved, is extremely important if we are to save biotechnology, in its broadest applications, for the poor, so that public institutions in developing as well as industrialized countries, can *harness its power for good.*

As a whole, the new, well-documented review paper of Adenle [326] delivers overwhelming evidence that GM crops are urgently needed in the developing world:

- The world needs fast and reliable solutions to fast growing population and the problems of hunger, malnutrition, ravaging diseases, poverty and global warming crisis. One of ideal technological innovations such as GM technology can be part of solutions to these problems. It is imperative to understand that GM technology cannot establish its ground if continuously faced with the baggage of constraints as discussed above. Moreover, it is not surprising to gather from a variety of literatures that most developing countries lack capacity building and still struggling with the establishment of biosafety system that can facilitate GM field trials and commercial release of GM products. Some of the challenges associated with the development of modern biotechnology still boil down to the fact that individual country government and international organisations have not clearly identified a coherent strategy and enabling policy instrument to deal with the problems. While some progress have been made on GM technology in terms of research and development, capacity building, and biosafety regulation in developed countries and a few developing countries, concerted effort is still needed to make it an accessible technology for every country. [326]

The Dispute Between Scientists and Opponents Today

The Role of Some Activist NGOs in the Debate

There is a continuous need for dialogue with regulators, the public, and specifically consumers, since the new technology emerging from modern life science is affecting all aspects of human life, including food, reproduction, etc. We do have an unfortunate trend toward irrational and antiscience argumentation in the GM crop dispute as clearly diagnosed by [327] in his book "The March of Unreason"), see also [328, 329].

This said, we should not create misunderstandings. There is no room for appeasement politics today when it comes to the activist NGOs like Greenpeace and Friends of the Earth, or websites like the Institute of Science in Society (I-SIS) and GM-Watch. Those professional organizations have proven repetitiously not to be interested in peer-reviewed science in a debate on the science and the sociocultural issues. They rather rely on unconfirmed reports in order to follow their own ideological and commercial interests. Any rational discourse with such organizations would be very welcome, but needs to be based on the latest peer-reviewed science. Their usual tactics is to appeal on fear. A good example from Greenpeace has been described on the EFB forum website http://www.efb-central.org/index.php/forums/viewthread/13/ about baseless accusations that 1,600 sheep have died from feeding Bt cotton leaves. A critique on the distorted picture on Indian cotton cultivation by NGOs is given by Herring [330] with lots of figures, facts, and extensive documentation.

Another blatant example of junk science has been launched recently by Greenpeace on You Tube "Genetic engineering: The world's greatest scam?" http://www.youtube.com/watch?v=1H9WZGKQeYg full of misinformation and hatred against multinational seed companies.

We are also confronted with violence – activities clearly documented and justifiably named and pursued as terrorism [331]. Also, in Europe, there are regularly occurring field destructions [332], which hamper seriously biosafety research – what an irony! Eco-terrorism is not confined to Europe, problems of such kind are very real also in the USA [333]:

According to the Federal Bureau of Investigation (FBI), the Earth Liberation Front, together with its sister organization, the Animal Liberation Front (ALF) has committed from 1997 to 2003 more than 600 criminal acts that have resulted in more than $43 million in damages. Moreover, attacks have been perpetrated in virtually every region of the USA against a wide variety of targets.

Recently, Greenpeace destroyed government field research in Australia [334] and defended the act of eco-terrorism with very thin arguments – and promptly lost lots of supporters and sympathy: Even

some old friends and supporters of Greenpeace (but not all) distanced themselves from the action: [335]. A list of field destruction actions in Europe has been compiled by Marcel Kuntz [336]. This list, far from being complete, demonstrates that activists have lost their moral compass in recent years: [337, 338].

One of the best rebuttals of cheap anti-GMO propaganda coming in attractive book editions, widely distributed in international events by the author Jeffrey Smith [339, 340] has been published on the Internet by Bruce Chassy http://academicsreview.org/reviewed-content/genetic-roulette/. It is actually a scientific comment, section-by-section, based on the best available peer-reviewed literature.

More chagrin emerges from the mounting pressure from within the academia, where, for instance, German university leaders in Giessen ordered to cease field research on GM crops which is unwelcome in the eyes of the extremists, [341] and there are serious complaints about the difficult atmosphere for biotech researchers in Germany [4].

Another symptomatic row is presently taking place in India, related to the approval of Bt brinjal, where activists are in a desperate attempt to stop the regulatory approval of Bt brinjal with outrageous and completely unfounded rumors like "GM brinjal will render the soil sterile," But contradictions have been posted as well: the most recent and comprehensive summary report published by Kameswara Rao [342], which is a review of massive evidence for the safety of Bt Brinjal and the detrimental heavy use of pesticides for the production of conventional Brinjal. It is ironic that one of the main arguments for proponents of the Bt Brinjal moratorium in India is now seriously questioned. There was the seemingly clear evidence on a crop biodiversity center for Brinjal in India, which called for extra protection of indigenous genomes. But recent extensive genomic analysis has clearly demonstrated that Brinjal is originating in Africa [343].

As an exemplary dispute, you can also follow the exchange of letters between the Public Research and Regulation Initiative (PRRI) and Friends of the Earth (FoE) [344]. Some of those anti-GMO activist groups get hefty funding from governments in the EU, as documented accurately by Andrew Apel and his GMobelus website: Europe's massive funding of world-wide activism. Compare also his recent article on the same subject, focusing on global aspects: [345].

The current set of arguments of GM crop opponents is often a mix of anti-American, antiglobal, postmodern, and even antiscience notions, [346], a strategy which has now been taken over very successfully by NGOs like Greenpeace and Friends of the Earth as global actors. These leading protest forces have helped, particularly in Europe, to build up a postmodern negative picture of biotechnology as a whole [347]. In this light, it is easy to act as "opinion leaders" with pseudoscientific arguments. The feedback mechanisms through the media and a network of citations of all the flawed stories make it possible for the global opponents to maintain confirmation of negation mechanisms. We are in a situation where the opponents already try to claim victory, penetrate highest political levels in governments and international organizations like the United Nations, some produce strikingly flawed reports on GM crops.

An analytical article about media and NGO activities in New Zealand has been published by Motion and Weaver [348]: by attracting media attention through dramatic protests, Greenpeace risks to jeopardize its reputation. The abstract reads:

> The challenges of attracting positive media attention are likened to a contest in which various organizations attempt to promote and circulate their version of events; however, this is particularly difficult when attempting to circulate less established, unpopular or critical knowledge. Although complying with, and managing, news values is an important starting point, the need to move beyond news values to consider the commercial values and realities of media organizations is highlighted. In this paper, a case study is undertaken of the Greenpeace media relations in New Zealand when a proposed controversial expiry of a moratorium to release genetically modified organisms into the environment. The predicament for Greenpeace is that in attracting media attention through dramatic protests it risks jeopardizing its reputation as a credible news source that can influence the framing of news stories. Insights are offered into the need for organizations to understand and manage the story or knowledge to be circulated and comply with contradictory news values.

Related to this paragraph on NGOs, it is necessary to write a word on the press: Newspapers and other media usually are mirroring what is important in the public debate, and the NGOs are clever in manipulating both the public and the press, after all, it is easy to provoke with fear and scaremongering, and the majority of journalists of all calibers are also committed to their own product, position, and its commercial situation.

A classic example is the coming and going of the Frankenfood Myth, see Fig. 3 and http://en.wikipedia.org/wiki/The_Frankenfood_Myth. Interestingly enough, this myth had its sharp peak in the press statistics around 1998 (see Fig. 3) and since then it has vanished from the headlines [104] as a major buzz word.

Those mechanisms have been precisely described by Burke for the situation in Great Britain some years ago [349]. But it is also clear that in the last 5 years more balanced voices appeared in the press, although there is no room to extend this topic here, just one recent example from the London Financial Times may suffice [350].

The GM Crop Battle, the Dispute Among Scientists, the Use of Strong Language

First, let us not forget some words of Antony Shelton [291], the most important words can translate into a slogan: "Quality of science must back up personal opinions," the abstract reads:

> In agricultural biotechnology there are roles and responsibilities of scientists, scientific journals, the public media, public agencies, and those who oppose or advocate a specific technology and serious consequences for science in general when those roles and responsibilities go awry. Scientists may feel the pressure of competition, especially in an academic setting. Personal views may continue to decide which issues one will work on, but the quality of science must back up those personal opinions. Common sense tells us that scientific inquiry and the publication and reporting of results to the scientific community and general population should be performed with high standards of ethical behavior, regardless of one's personal perspective on agricultural biotechnology.

One of the arising problems is that there has been recently a tendency to mollify peer review for the sake of politically correct so-called critical views of genetic modification of crops, with some blatant examples of flawed pseudocritical papers having passed for publication in highly respected scientific journals – a few examples have been commented by [351]. Some of those papers just passed due to flawed peer review, others passed despite rejection by some peer scientists, obviously for the sake of public debate (and for the promotion of the journal), see as an example the rather thin justifications of the editor in chief of Lancet Richard Horton to go ahead with the publication of Pusztai's rat experiments [352–356]. For more details about this controversy, see in ASK-FORCE on Pusztai [357], it is an anatomy of the case in 46 pages on the Pusztai affair, which had a big influence on the regulatory climate on GM crops in Great Britain and the world.

It is only between 2005 and 2011 that a certain fatigue of new negative arguments against GM crops is developing, and it is interesting to note that opponents, lacking real negative health and environmental effects, now shift their emphasis on negative arguments in socioeconomics. There are hardly any new issues in food safety and environmental impact to be dealt with in the last few years. This might also be the reason why in a desperate routine of repetitious "negative," GM crop stories get into journals, often also on rehashed events which have been clearly rebutted scientifically many years before. Those "news stories" often pass uncontested and get printed in "news" media due to a mix of short memory effects of uninformed editors and readers of all kind, or worse, they are purposefully repeated by activists counting on short memory of press and public.

A strange effect should also be mentioned that scientists, who defend good science in biosafety research, sometimes get blamed because they use straightforward language when criticizing flawed papers. A paper on such debates has been published by Nature [177], see the comments in a contribution of ASK-FORCE [178] on a paper on aquatic organisms supposedly harmed by Bt toxins of GM maize by Rosi-Marshall [191] and [192]. There are several controversial hints in this Nature story put forward by science journalist Emily Waltz, who is neither specialized nor experienced in the hot scientific regulatory debate on GM crops, suggesting that to criticize flawed papers

with "strong language" is detrimental to the progress of scientific research. This statement was supported by interviewed writers such as Ignazio Chapela (famous for starting the controversy of the Mexican gene flow of transgenic maize with a letter to Nature [358], which later turned out containing insufficient evidence for publication [359], see the latest summary in [360]. Another interview Waltz conducted in the cited *Nature* piece with David Schubert, who tries as a pharmacist to give advice in biosafety rules of GM food, and with his strong anti-corporate mood publishes fraud accusations against pro-GMO scientists [361]. Both interviewees Chapela and Schubert defend independent scientific whistle blowing, but themselves they have a proven negative agenda about GM crops, see more controversy papers: [295, 362, 363]. In the meanwhile, several letters to the editor of Nature have been written commenting the feature of Emily Waltz in Nature, they are all cited in [178], the majority is not supporting her thesis.

Incidentally: Strong language has been used before in the history of science, remember some really bitter and hefty disputes about the history of discovery of the double helix structure of DNA between Watson and Crick [216], who later made their peace again.

Other numerous examples of a fight out in the open are documented about evolution when Darwin proposed his revolutionary ideas. Two citations of strong language may suffice: in a debate on natural selection [364] writes on a dispute with William Bateson:

- By these admission almost the last shred of that teleological fustian with which Victorian philosophy loved to clothe the theory of evolution is destroyed. Those who would proclaim that whatever is right will be wise henceforth to base this faith frankly on the impregnable rock of superstition and to abstain from direct appeals to natural fact.

Another clear example of sharp and relentless scientific controversy on evolutionary biology with strong language has been described in detail by Strick [365], among the numerous juicy examples:

- His [Bastian's] tone was sharp in response to Huxley's public accusations that his technique was sloppy (a much more high-powered attack than Huxley ever adopted in private when attempting to correct young scientists). Huxley replied with an equally sharp tone, now saying sweepingly that "what Bastian got out of his tubes was exactly what he put into them," *i.e.* contaminants.

And one last word about strong language: The word "abuse" has been printed by Nature in the Battlefield paper [177] very prominently in the subtitle, when attacking a group of authors including me who criticize flawed papers in the GM crop debate with blunt, but still polite words – what an irony! – And to be quite clear, no complaints from my side. . . .

Negative Effects of Modern Breeding Methods in Food and Environmental Safety do (or Should) not Pass Strict Scientific Procedure Rules and Peer Review or They Are Based on an Unscientific Focusing on Transgenesis Instead on Management Mistakes

If researchers would follow strict procedural rules, the world of scientific biosafety debate would be far less complex, here are a few papers standing for such in fact uncontestable rules: [168, 260, 261, 267, 312, 366–369]. It is a fact that for some years basically no new arguments against agricultural biotechnology (in particular clearly related to transgenesis) on an agronomic base can be put forward for the most widespread crops, which have run through multiple regulatory processes in many countries.

This does not mean that transgenic crops are completely free of problems, but, in fact, it is that in comparison with conventional crop problems these are minor and manageable in a more efficient way. One of the basic mistakes of GM crop criticism is the unilateral focus on the risks of transgenes inserted, instead of comparing, in a fair and scientific (holistic!) way, with conventional cropping [370].

Still, a growing number of herbicide tolerant weeds are emerging: [371–374]. Powels [375] rightly points to the monotonous fields of glyphosate-resistant soybean landscapes, where the herbicide-tolerant weeds emerge more rapidly:

- Indeed, in spite of longterm use, the evolution of glyphosate-resistant weed populations in non-GRC, burndown systems has been very limited. Thus, functionally competent gene traits endowing glyphosate resistance are relatively rare and not easily enriched in

> plant populations [376], [377]. This is why glyphosate is a remarkably robust herbicide from a resistance avoidance viewpoint. However, as reviewed above, it is clear that, where there is very intense glyphosate selection without diversity, glyphosate resistant weed populations will evolve. In particular, the evolution of glyphosate-resistant weed populations is a looming threat in areas where transgenic glyphosate-resistant crops dominate the landscape and in which glyphosate selection is intense and without diversity. [375]

But it is also a fact that the emergence of glyphosate-resistant weeds is happening on a much slower pace than that of conventional herbicides [378].

Some critical science journalists question the strategies and behavior of the global opposition players. In a kind of last bid, questionable reviews are published, either containing lots of negative *assumptions* [379] or wrong toxico-analytical concepts resulting in an exaggerated risk assessment for nontarget insects as the lacewings as promoted by Hilbeck et al. [380–382] and contradicted clearly in Romeis [383]. Other examples of questionable eco-toxicological conclusions have been drawn by producing or reviewing flawed data or statistics, or drawing questionable conclusions, see the debate on Ermakova's flawed rat experiments: [384], more details in a contribution to the ASK-FORCE [385]. Typical other examples recognizable on filtered citation lists are Dona et al. and Séralini et al. [199, 386]. Séralini conducted his experiments in disrespect of the internationally approved rules of biosafety experiments established by the OECD [387, 388] and also avoided the citation of certain contradicting peer-reviewed references. Many of those papers have been or will be treated in ASK-FORCE [389], where you can read about new or recently updated ASK-FORCE contributions, for more details see section ASK-FORCE Organization and Related Websites.

It also must be said (remember Saner's statements at the beginning of this section) that vested interests can be spotted with some biosafety researchers, who are in need of research grants and thus paint a negative picture on biosafety; they symptomatically have difficulties to distinguish between the "nice-to knows" and the "need-to knows." Example: see the ASK-FORCE contribution [178] on the publication of [13], a paper which is flawed in several ways. It has been completely rebutted by Shelton et al. [14], the questions asked in the Lovei paper are irrelevant for Bt maize cultivation, since the Bt-toxin-technology is overwhelmingly beneficial for majority of nontarget insects [390–394]. One of the major flaws of the Lovei paper is that they used low quality prey for their laboratory feeding studies. A thorough analysis of risk assessment research has been recently published by Raybould [261]: We need to carefully distinguish between basic ecological research and purposeful and targeted risk assessment research which concentrates on the real agronomic risks and needs [395, 396].

The question and negative answer given in the letter of the Public Research and Regulation Initiative (PRRI) to the Secretariat of CBD [397] is fully justified, *and PRRI stands ready to expand on the points made in this letter.*

- 1. Are there LMOs or traits that have caused adverse effects?

  No. Since the first application of genetic modification in the 80s, many thousands of field trials have been conducted with GM organisms (to date mostly plants), and since 1996 many hundreds of millions of hectares have been planted with GM crops by many millions of farmers and consumed by hundreds of millions of consumers in developed and developing countries, without any verifiable reports of adverse effects on the environment or human or animal health.

  In fact, taking a broader look, experience with those GM crops has shown environmental and socio-economic benefits in terms of increases in yield, significant reductions in use of pesticides, fossil fuels and soil erosion, less mycotoxins in grains, as well as increased farmers health and income.

Final remarks: Coming back to the first statement of Saner [120] given under General Views on the Dialogue Related to Regulation of GM Crops, value-laden scientific activity cannot be avoided, but minimized – if you refrain to work with flawed data, with filtered citation lists, and with reviews pontificating on negative assumptions. The only remedy is to work with high-quality data produced in a methodologically transparent way following international agreement.

It is appropriate to end this rather pessimistic section with a positive note, not free of irony:

As Gupta [398] recently stated, there is hope that the introduction of strict biosafety rules in the Cartagena Protocol, originally aiming at a slowing down or even at stopping the transboundary movement (and indirectly development) of GM crops, now seems to turn into its contrary:

- Through analyzing the dynamics of GMO-related information disclosure to the global Biosafety Clearing House (BCH), I argue that the originally intended normative and procedural aims of disclosure in this case to facilitate a GMO-importing country's right to know and right to choose prior to trade in GMOs are not yet being realized, partly because the burden of BCH disclosure currently rests, ironically, on importing countries. As a result, BCH disclosure may even have market-facilitating rather than originally intended market-regulating effects with regard to GMO trade, turning on its head the intended aims of governance by disclosure.

Debate Improvements: What can we do to Enhance the Situation?

Foremost, it is important to *shift from pro-reactive to proactive mode*. This does not automatically mean to filter away negative views on GM crops and to organize a eulogy on the benefits, the pro-active mode should actually engage a new mode of debate, which is more discursive, more structured and definitely concentrates on a solution-oriented decision-making process. It is time for action – as far as a strict scientific view is allowing this. There are several websites working hard on sorting out the strictly science-oriented messages in biotechnology, as mentioned below. We should not, as it often happens, in our struggle against the negative pseudo facts focus on the risk alone and thus trap ourselves in a negativistic perspective.

Rather we should address in a balanced way the obvious (or lost) benefits as well. But this alone will not provoke a turnaround. This shift must be embedded in a discourse with concerned people and organizations and it must clearly oppose untruthful strategies of the global protest corporations and thus also refrain from using the same countertactics. One of the appropriate organizations for this activity will be the two platforms: (1) Public Research and Regulation Initiative PRRI www.pubresreg.org run by public researchers and (2) also the European Federation of Biotechnology http://www.efb-central.org/, so that public science will get a more important place in the international regulatory debate (but also where private seed companies are not fundamentally battled in a naïve neo-Marxist scheme). In many meetings strictly based on science and organized by PRRI, both platforms are well received. The project outline can be described as follows:

ASK-FORCE Organization and Related Websites

There is a flood of papers which cast doubt on the GM crops already regulated in many countries. Most (if not all) of these papers are written in a bad quality, either with flawed methodologies not internationally agreed upon, or with conclusions which are not supported by the data [13], rebutted by [14], details see in [178]. There are also many reviews published in a scientific style, but unfortunately either with a strongly biased set of references or with unsupported assumptions and doubtful conclusions – contradicted by peer-reviewed publications often not cited. In some cases, the flaws are more hidden: Experimental data are achieved on clearly theoretical schemes, working with outdated Bt maize and nontarget butterflies which have in their biology, in nature, no connection to maize fields: [399]. It is therefore important to set the record straight and to try to rebut at least the most important and blatant cases.

Within an EU project with Marc van Montagu and Piet van der Meer, which has been granted to PRRI, a blog was launched with the name ASK-FORCE on the PRRI website www.pubresreg.org with the secretarial help of Kim Meulenbroeks (until 2008) and presently Zuzana Kulikova. A list of about 130 items [400] has been compiled with international help and will be entered step-by-step in the grid of the following six sections. (1) General (2) Human and Animal Health (3) Environmental Safety (4) Agriculture (5) Public Perception (6) Developing Countries.

Up to now, 11 contributions have been published on the Internet; for links and contributions see [389]. These were reviewed by the experts of the steering committees of Public Research and Regulation Initiative and the European Federation of Biotechnology,

some also by the experts united in the blog community of AgBioWorld http://www.agbioworld.org/. All three lists contain some of the best specialists on green biotechnology from all around the world for reviewing and commenting.

In order to become more proactive, we need to develop forward-looking strategies. It is up to the scientists to ask questions to the opposition, and in particular to the professional distorters of the scientific facts. This must escalate into public campaigns if (what is to be expected) those specific questions are ignored. Carefully built contacts with science writers are important here, as a help for networkers a selected list is given here http://www.ask-force.org/web/ASK-FORCE-Summary/Contacts-ASK-FORCE-2011.pdf

Long-Term Discourse and Decision-Making Processes

Let me first be quite clear that I think a dialogue with the professional protest corporations is, as a rule, a waste of time (specifically Greenpeace and Friends of the Earth, not to mention some other organizations). Their only interest is to keep the pot cooking and make sure that the population remains in a state of fear. They should be addressed with a confrontational strategy, which is included in ASK-FORCE. Often such NGOs get the willful help of the press, which acts according to the old proverb (Macbeth, Shakespeare) "evil always fascinates – goodness rarely entertains" [401], see also the arguments produced by Andrew Moore [402]. While some press products concentrate on mirroring public concerns, a press more or less close to boulevard strives to foster its marketing with the help of sensational headlines, creating stories which sell better, but indirectly they are exacerbating the problems. We are also not going to talk about a special discourse, as described by Erjavec [403], related to the politics of the EU commission.

Nevertheless we have to address all segments of the public with its concerns, feelings, and interests. And the discourse we are going to concentrate on is solution oriented. This should be done according to the discursive rules of the management strategies of the second generation, the *Systems Approach* (see under The Second Generation Systems Approach as a New Decision Making Process). As a basic reference with description and citations, see the classic book of Churchman [79]. If we follow some ground rules, this should not be too complicated.

The Second-Generation Systems Approach as a New Decision-Making Process

Instead of making questionable concessions (example: "let's not talk about transgenic crops" as often done by Nestlé and Unilever, with notable exceptions [404] within these two companies!), the dialogue should be organized in an atmosphere of "Active Listening" [405] and understanding in which, apart from the strict rules of scientific argumentation we should send signals that the new technologies also trigger socioeconomic and cultural feedbacks. This will be the key to solve *Wicked Problems* [406], which contain also sociocultural elements besides a set of hard, often contradictory facts [122]. In his usual cynic precision, George Bernard Shaw defined the ultimate problem in the dialogue between scientists and lay people: "Every profession is a conspiracy against the laity."

The new discourse is not about the usual stakeholder meetings; rather it is about instigating modern planning processes of the second generation in evidence based but open ended decision-making processes. This *Systems Approach of the second generation* contrasts to linear planning with predetermined targets and dominating deontic thinking (e.g., of the industrial corporations and government agencies), it contrasts also to *the Systems Approach of the first generation* (e.g., Apollo moon landing with clear target).

The Rationale of New Management and Decision-Making Processes

> ▶ Some problems are so complex that you have to be highly intelligent and well informed just to be undecided about them. Laurence J. Peter [407]

These new strategies should dissolve the traditional stakeholder concept in favor of a much more efficient system respecting *different kinds of knowledge* and other rules (such knowledge differentiation is also known from learning processes, which are related to our decision-making dynamics [408].

There are more practical reasons to employ into the Systems Approach and its concept of different kinds of

knowledge, as Zwart [409] rightly emphasizes: Ever since we have realized that the low number of human genes (approximately, 22,500) cannot be interpreted as a narcissistic offence, since organisms are so highly complex, including the emerging consciousness of our human brain, genomics takes us now beyond a genetic deterministic understanding of life, this must have consequences on societal research and debate as well. Policies for self-improvement will increasingly rely on the use of complex interpretation. *Therefore, the emphasis in our discourse must shift from issues such as genetic manipulation and human enhancement to issues involved in governance of novel forms of information.* The same can be said on the side of agriculture. Ikerd [410] develops with the means of the systems approach a more holistic picture of agricultural management.

Fairclough [411] as a linguist gives an in-depth and critical analysis on discourse related to globalization with lots of facets, and again with a totally different set of terminology, he also presents negative examples of discourse. Objectivism treats globalization as simply objective fact, which discourse may either illuminate or obscure, represent or misrepresent. In the Churchman systems approach, there is no such thing as an objective approach, rather it is objectivation. Ideologism focuses upon how particular discourses of globalization systematically contribute to the legitimation of a particular global order which incorporates asymmetrical relations of power such as those between and within countries.

Scoones et al. [412] come to similar conclusions as the Churchman school, but this time related to agricultural policy, the paper explores the national and transnational character of mobilization against GM crops in India, South Africa, and Brazil in the 10-year period up to 2005. The paper argues for a better understanding of national political and economic contexts which must be taken into account, alongside on how the GM debates articulate with other foci for activism and the complex and often fragile nature of alliances that make up activist networks. It is important to understand that the debate about GM crops has become a much wider one: about the future of agriculture and small-scale farmers, about corporate control and property rights, and about the rules of global trade, see also the new report of the Royal Society [18]. In sum, a debate should not just focus on the pros and cons of a particular set of technologies – after all, they have proven safe – it is more about politics and values and the future of agrarian society. Again we see the plea for the complexity of "*wicked problems*" to be solved.

The downside is that those planning processes of the second-generation are time consuming and need a careful and tedious procedure in developing the most important and difficult *zero-step* – before such decision making can be started. It also implies an exchange of knowledge between the parties beforehand, in order to minimize *hidden agendas*. It also must be emphasized that those decision-making processes do not lead necessarily to a predefined goal, they are often *open-ended* and demand flexibility among the discourse participants, who need to remain open-minded.

The more questions we ask the more answers are possible and vice versa. Limitations of technological solutions are always hidden in the open ecological and social systems: Just compare the (in)famous case of DDT sprayings in the past [413–415]. Today, it is clear that with linear planning, DDT has been banned for ecological and health reasons, not considering the wider argument field of malaria prophylaxes. This inconsiderate DDT ban has caused millions of malaria deaths in Africa. Today, reasonable domestic use of DDT has again lowered the malaria threat measurably. Constraints in possible secondary effects in ecology should be examined carefully. This is well demonstrated in the case of the Monarch larvae being killed by Bt-Maize-Pollen, the result of a laboratory study published in Nature [416] where the subsequent press interpretation got way out of proportion – even though the author Losey himself warned about the limitations of this small lab study. Would researchers have asked the farmers, they would have been able to say that feeding time of the young larvae do rarely overlap with the time of pollen shed of maize, and that the plants the Monarchs are feeding upon are fiercely fought as a weed. Subsequent field studies revealed that there is no problem arising from extensive Bt maize planting for the Monarch larvae [12].

In order to tackle wicked problems, you need to go through *an extensive process of argumentation*, also called objectification, not to be mixed up with an "objective approach" to the problem.

There is rational planning, but there is no way to start to be rational: One should always start a step

earlier, since there are important trends and facts which will make straightforward rational thinking and acting in solving wicked problems useless. It is not the theory component, but rather the political component of the knowledge, which determines the vector of the action. This is the *zero-step* so important in the publications of Horst Rittel [121, 122].

As an example: The fact, that experts can be wrong and farmers know better in certain situations in agriculture because they are better observers out in the field and because they are very experienced in traditional knowledge [417].

The knowledge needed in solving wicked planning problems is not concentrated in a single head. It is absolutely essential to let all partners be involved in the problem solution process, which includes part of the population (mainly farmers' organizations and consumer organizations), the Governmental Regulators, the Non-Governmental Organisations, the Life Science Companies, and the Scientists. There is no monopoly of knowledge. Having illustrated the difficulties in solving wicked problems, we need a new approach in problem solving, in order to avoid the pitfalls of ignoring bottom up feedbacks.

You only can keep to this rule if you are also following another important rule. All partners in the planning process have to avoid hidden agendas, which is certainly eased by a minimum amount of respect paid to each other partner. Nobody should be criticized for speaking up in his own interest.

A caveat: It would be naive to just believe in the discursive capacities of the civil society, contrary to what Gerhards [418] has shown – that Habermas' support for the discursive model is based on the assumption that actors of the civil society argue much more discursively and on a higher level of rationality than other collective actors do. But empirical results show that actors of the civil society are, maybe, even less discursive than other actors.

It is primarily the paradox of rationality which has been severely underestimated in the systems approach of the first generation when tackling *wicked problems*.

How to Solve Wicked Problems in Biotechnology and the Environment What we need in such cases is an action-oriented approach. Risk Assessment and Management must be seen as a planning strategy of the second generation in developing a professional framework for *decision making*.

Strategies have to be developed to recognize the consequences of our doing on one side, and to specify our knowledge on the other side. This knowledge has to be gained step by step and case by case. If we want to clearly distinguish our present state knowledge from appropriate decisions to be made *not* based on our views and opinions, we need to go through the following steps:

- What is the problem?
- What do we want?
- What are the alternatives?
- How do we compare them?
- How can we reach the solution?

All participants need to keep in mind that there are *various types of planning knowledge* (arranged according to the five questions asked above).

Examples given here are lumped together as simple keyword illustrations, taken out of their context in real planning examples, and they cannot be regarded as an example of a realistic situation; this would be exactly the task of a planning process of the second generation.

Factual knowledge is the knowledge of what actually happens (quantitative data or empirical, observational data). Gene flow species by species/region by region/facts about insect resistance in agriculture.

- *Deontic Knowledge*, the very important knowledge of what ought to be. The knowledge about new crops which enhance agricultural production/new agricultural techniques to avoid erosion/new biological approaches to fight insect pests etc.
- *Explanatory Knowledge* explains why things are so or why certain effects will happen. Here, you already start to determine the direction of the solution. The way Bt proteins are acting on specific pest and beneficial insects/what are the main reasons of unwelcome erosion effects/mechanisms of vertical gene flow/mechanisms of resistance development.
- *Instrumental knowledge* on how to steer certain processes, on how to achieve certain goals, knowledge which needs to be balanced against regulation and safety. The way how to build Bt and other genes into crops and how to stabilize them/how to avoid

vertical gene flow/how to avoid unwelcome soil erosion/how to avoid early upcoming pest resistance.
- *Conceptual knowledge* which would allow avoiding conflicts before they pop up. This is the knowledge about complex situations, taking into account all previous kinds of knowledge and also weighing them against arguments coming from open ecological and societal systems. Concepts about transgenic crops compatible to the ideas of a sustainable agriculture. Lawyers and judges also may work with this kind of procedural knowledge.

You need to go through an *extensive, time-consuming process of argumentation*, also called objectification, not to be mixed up with an "objective approach" to the problem. The hopes of this process are:

- To forget less, to raise the right issue
- To look at the planning process as a sequence of events
- To stimulate doubt by raising questions, to avoid short-sighted explicitness
- To control the delegation of judgment. Experts have no absolute power; scientific knowledge is important, but always limited.

There is no such thing as "scientific planning."

- Solving practical problems as to develop sustainable transgenic crops cannot be dealt with by "scientification of planning." Dealing with wicked problems is always political because of its deontic premises (means that you have to involve knowledge what ought to be) and because we deal with traditional knowledge. Science only generates factual, instrumental, and in the best case explanatory knowledge.
- The planner (here the manager of an action plan) is not primarily an expert, but a *mid-wife of problem solving,* a teacher more than a doctor. Moderate optimism and careful seasoned disrespect, casting doubt is a virtue, not a disadvantage of an action plan manager.
- The planning process of wicked problems has to be understood as an *argumentative process*, it should be seen as a venture (or even *ad*venture) within a conspiracy framework, where one cannot anticipate all the consequences of plans.
- Systems methods of the *second generation* are trying to make this deliberation explicit, to support it and to find means in order to make this process more powerful and to get it under better control *for all participants.* Methods like the computer-based argument mapping systems of can be helpful [419].
- It helps making such processes more successful if they are conducted in the spirit of the *Symmetry of Ignorance* [420] – this is the secret of the active listening which often leads to acceptable outcomes and trust.

This seems to be a rather theoretical approach with lots of restrictive rules, but actually it is, on the contrary, an opening for much more freedom in dialogue. Also, it is more practical and efficient in creating results and contrasts with the traditional stakeholder concept where hidden agendas prevail in often disguised authoritarian structures. Such discursive processes are described in detail [80, 121–123, 421–425]. A comprehensive and voluminous monograph on risk-related debate methods has been published by Ortwin Renn [426], see especially the texts related to risk communication with essays 7 and 8 and section 8 on risk participation with numerous references, but notably lacking completely the papers on the "Systems Approach" of the Churchman/Rittel/Webber school.

In a French paper, the origin of negatively connoted words in the debate on GM crops like "contamination," "pollution," "Frankenfood," etc., Moirand [427] clearly reveals the links to negative events like BSE, dioxin scandals, and of course Tchernobyl, etc., thus explaining new words like "mad soya" and "mad colza" in the media. Moirand concludes that a new type of discourse is needed, but also Renn [426] does not refer to the very pragmatic and promising systems approach of Churchman and Rittel.

There are many more schools promoting discourse and new decision-making processes, also in specialized journals, only a few can be summarized here for space reason: [75, 76, 78, 84, 119, 120, 411, 427–441].

See Patrick Moore's practical examples of decision-making processes solving environmental and sustainability problems in forestry, consult his own website Green Spirit http://www.greenspirit.com/index.cfm. These processes need time. Patrick Moore [442–444] has gone successfully through such processes in the

difficult task of reconciliation between the needs of timber production and environmental constraint; he needed months of debate to come to reasonable decisions.

Another good example on how group discourses have good learning effects, has been described by Snyder et al. [258]: Although the US government has assured stakeholders of their safety, the EU continues to be an outspoken opponent. This can largely be attributed to a lack of trust in the regulatory process, and especially a cynical perspective on the underlying science and institutions that govern approval. Such disparities were illustrated in 2003 when the USA donated GM maize to aid African countries stricken by famine. Under purported EU threats, negative propaganda by NGOs, and stressing retaliatory trade sanctions, African officials refused the aid. An examination of this episode contrasts the potential discord between those affected and those who formulate government policy. Using resources from both sides of the debate, this scenario summarizes the pertinent issues regarding EU's refusal to the import of transgenic crops. A group discussion and debate protocol was developed for facilitating small group and entire class consideration of the scenario while strengthening student critical thinking skills.

It helps, if you prepare carefully scenarios before people start the process, a method which has been successfully applied to the reconciliation processes in South Africa after abolishing apartheid by Adam Kahane, one of the principal mediators [445]. He also followed another wise rule: Should only people participate in such processes who are part of the problem. Another excellent example of long-term discourse is described in many aspects by von Grebmer et al. [437]:

- By working collectively the process will be more open, transparent, inclusive and accountable, and sensitive to the normative dimensions of the issues critical to the participants. The themes and processes outlined in this article set the stage for the discussions, internally and between countries, that will shape the policies of agricultural biotechnology in the region. If the dialogue can frame the discussion and be enriched by the information generated from actions taken, it can sustain the interest and commitment of the stakeholders, and more successfully direct biotechnology toward reducing hunger and poverty in the region.

There are too many scientists remaining in the ivory tower, shying away from public debates. They fear losing their independence, a fear which is not just unfounded, but actually it is the contrary: remaining in the academic ivory tower means having lost your independence, since science is not an art per se, it is full of importance for society and humanity. A strong plea in this direction is coming from [446]. Although science should remain at the heart of invention and the drive to make our lives better, scientists should, instead of always having "the answers" ready, should not be afraid to engage in a contradictory evidence-based mode.

In one of the most successful examples of long-term discourse, the author participated as an invited expert in a public hearing in 2000. Strikingly, it was done without the theoretical load described above, but with lots of financial and logistic help from the New Zealand Government, in particular from the Royal Commission on Genetic Modification. A report was finalized after a 14-month inquiry into the risks and benefits of genetic modification. It heard from over 400 experts, including scientists, environmentalists, and ethical specialists. It considered more than 10,000 public submissions and heard the view of many others during a series of public meetings, hui, and workshops around New Zealand.

The Royal Commission's major conclusion was that New Zealand should proceed cautiously with genetic modification (GM) but not close the door to the opportunities offered by the new technology http://www.mfe.govt.nz/issues/organisms/index.html. The discourse is still continuing. Again, it is visible that the discourse is less confrontational and may lead to innovative solutions in the future [447]:

- The debate about genetic modification (GM) can be seen as characteristic of our time. Environmental groups, in challenging GM, are also challenging modernist faith in progress, and science and technology. In this paper we use the case of New Zealand's Royal Commission on Genetic Modification to explore the application of science discourses as used by environmental groups. We do this by situating the debate in the framework of modernity, discussing the use of science by environmental groups, and deconstructing the science discourses evident within environmental groups' submissions to the Commission. We find

science being called into question by the very movement that has relied on it to fight environmental issues for many years. The environmental groups are challenging the traditional boundaries of science, for although they use science they also present it as a culturally embedded activity with no greater epistemological authority than other knowledge systems. Their discourses, like that of the other main actors in the GM debate, are thus part of the constant renegotiation of the cultural construct of 'science'.

However, this process should not be mollified on the costs of hard science. The line between science and pseudoscience is often difficult to draw.

A Remark About the Psychology of the GMO Debate To be written in the next coming days.

It should also be possible to think and act in relation to the reconciliation of science and spirituality, since it will be an important element besides the ratio of science, the ethics of our societal activities, and the emotional elements in human life. But it will be difficult to separate the cheap esoteric chaff from the precious seeds of true spirituality, as Helmut Reich's writings demonstrate [448]. We must endeavor new fields of thought, as done by Papazova Ammann [449], a Bulgarian-born Swiss philosopher with roots in the schools of Muntjan and Rittel.

- What do we need as visionaries: Progress or Development? This is my question today, as I deal with the topic of Biovisionaries here in the Library of Alexandria. I ask this question because I am convinced that we need to build a new culture of questioning. We need a culture orienting itself by authentic questions. How can we develop taste and the ability to distinguish between those questions which are cognitive, statement-oriented and those which are authentic, close to life and to people? What is more important: cognizance or decision for action? How can we move between Statements and Questions? Statements reflect the need to understand the world. But they are the result of past experience and are often contained in frameworks which are coined by society. They may even protect old routines which hinder innovation. Questions, in contrast to statements, can transform our judgements and prejudices. Questions give birth to energy for new orientation, for a more conscious future. This orientation towards the future, towards vision provokes those choice-questions, and they alone will open the way for an urge to change the world. Visions need people who are free! The quality of freedom is inherent in the question. We must strive for this quality through choice-questions. If we cannot befriend these choice-questions with science, it will disengage from the questioners and will not be human science anymore. Thus we need a new humility of thinking – as it has been wonderfully defined by the German philosopher Heidegger: "The question is the devoutness of thinking".

Conclusions Only a multifaceted dialogue over a considerable time span will lead to success. The Internet scene is developing fast and new communication software tools are available now, so careful scrutiny for such a network of networks need to be done first, and the big players like Google and competing networks should be consulted as well.

Personal experience in dialogue with many networkers reveals that sometimes important networks are only known in specific clusters, these lacunas should be closed for many reasons – see section Illusions and Realities on Educational Effects in the Debate, the Dialogue Between Science and the Public. Knowledge exchange, jumping over national fences, and coordination will be a follow-up effect, without even declaring it to be the goal of such activity. As for now, this is just an idea and needs to be discussed with Internet and website specialists. After all, the leading webmasters and coordinators agree that it is time to *enhance collaboration through better communication.*

ASK-FORCE can contribute to this process in making sure that professional peer-reviewed risk assessment papers are fed into the dialogue processes and are ideally fed into a life decision–making process with relevant participants.

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Grain Quality in Oil and Cereal Crops

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Article Outline

Glossary

Cereals Monocotyledon plant grains that accumulate starch as the main storage substance for subsequent germination. Two types have been distinguished – cereals that contain gluten and are used for bread-making (wheat, oats, barley, rye) and cereals that do not contain gluten (rice, maize).

Genotype × environment interaction Relative changes in genotype performance when grown under different environments.

Grain development Structural and functional changes that occur in the fertilized flower producing a mature grain capable of germinating.

Grain growth Irreversible increase in grain weight and size caused by cell division, expansion, and reserves accumulation.

Grain quality Group of grain characteristics and measurable attributes (objectively or subjectively) to meet the clients' requirements (i.e., customer, industry, consumers).

Oilseeds Dicotyledon plant grains that accumulate oil as the main storage substance for subsequent germination. Oilseed crop seeds (sunflower, rapeseed, ground pea) are composed of 40–50% oil and 20–30% protein while proteo-oil crop seeds (soybean, lupine) comprise 15–30% oil and 30–40% protein.

Photoassimilates Carbohydrates (sugars, starch, or fructans, depending on the species) synthesized by the green plant parts and translocated to actively growing organs, like grains. Photoassimilates may originate from current photosynthesis or reserve remobilization.

Source–sink balance Quantitative relationship between plant photosynthetic capacity (source) and number of organs under active growth (sink) that are sustained by the former.

Plant stress Changes in plant metabolism in response to environments that endanger plant survival or hinder reaching maximum reproductive capacity.

Definition of the Subject

Grain quality is frequently regarded by agronomists and breeders to be as important as yield. Quality characteristics are the reason why only few plant species are used to satisfy most human requirements for food and fiber [1]. Grain quality comprises a group of characteristics that collectively determine the usefulness of the harvested grains for a particular end use. Therefore, to breed and manage grain crops to achieve a specific quality standard and to be able to predict the quality of a particular crop in a particular growing environment is rather important. Achieving this objective is dependent upon the knowledge of the factors modifying grain composition, and consequently grain quality.

As grain markets have become more specialized, there is a growing pressure on farmers to produce grains with greater uniformity and with certain characteristics [2]. Appropriate husbandry to obtain grains with high and stable "quality" will likely be of increasing importance in achieving economic benefits. It is well known that grain quality is modified by the environment and the crop management practices used by farmers. However, the strategies and tools required to produce grains with certain quality characteristics

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are not as well established as the ones for achieving high yields. In this context, improving the understanding of the factors that determine grain quality has become increasingly important.

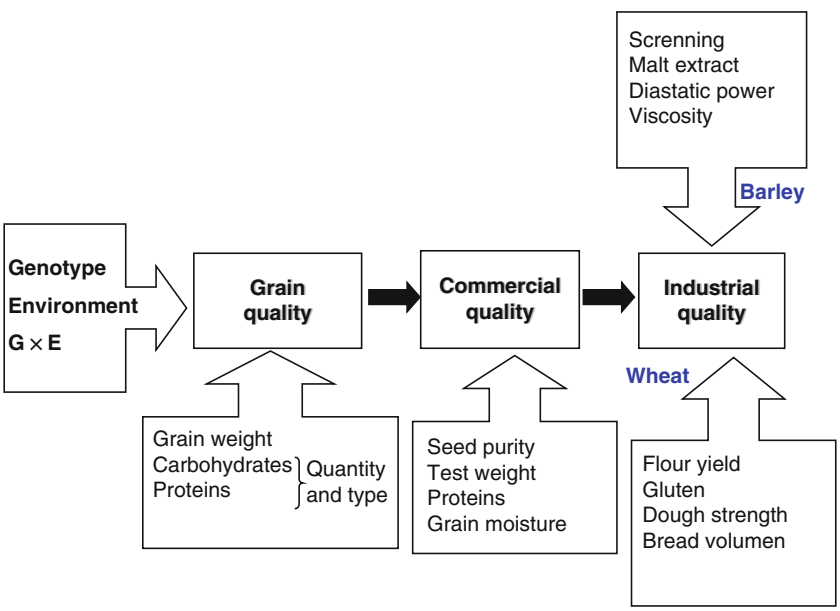
Grain Quality: Concept and Importance

In field crops, the quality of the end product is related to the composition and structure of the seed at harvest maturity. Seed composition and structure at harvest are determined by the genotype, the environment, and the crop management practices used during the crop growing cycle. It is not possible to propose a unique grain quality definition for any specie because it changes depending on the product end use. There is a proper criterion on the concept of quality for each specific end use and for each stage of the commercial chain in every crop (i.e., from harvest in the field, through grain dealers to the industry, Fig. 1). In this context, quality will be considered in relation to the criteria used by those involved in the various aspects of growth and utilization of the grain. As an example, for wheat and barley (Fig. 1), grain quality at the moment of harvest in the field is related to grain size (and weight) and the carbohydrates and protein composition. When the grain is sold to the grain dealer, seed purity, test weight, grain moisture, and protein percentage are the main characteristics that are taken into account for the prize (Fig. 1). After this stage, other attributes may be relevant and they will depend on the involved industry. For baking industry, flour yield and dough strength will be of maximum importance in wheat, while barley for producing beer will take into account the screening percentage, malt extract, and diastatic power, which in turn is related with nitrogen content.

This article aims to summarize key elements of grain structure, grain growth, and synthesis of major grains components in field crops in order to highlight the main attributes which modify grain quality.

Grain Structure

Harvested cereal and oilseed organs may comprise true seeds (soybeans, rapeseed) or fruits (seeds and maternal-accompanying structures, like sunflower achenes or wheat, barley, rice, maize, and sorghum caryopses). Seeds develop from fertilized ovules and consist of three genetically different tissues: (a) the embryo developed from a zygote (diploid, representing the next



Grain Quality in Oil and Cereal Crops. Figure 1
Schematic postharvest processing and storage of wheat and barley production and main quality attributes in each step

generation), (b) the endosperm (usually triploid), and (c) the seed coat formed out by integuments, representing the maternal tissues of the ovule [3]. The proportion of these three components differs in mature seeds of cereals and oilseeds; endosperm is preponderant in cereals while the embryo prevails in oilseeds. With a few exceptions, the development of the endosperm always precedes that of the embryo; and the seed coat development precedes both. These genetically different parts interact closely during development and germination, and recent studies demonstrate the complexity of the connections and regulations among the different seed tissues [4, 5]. After fertilization and seed setting, grains are the primary sink in the plant. Grain filling requires important amounts of photoassimilates supplied by the mother plant through actual photosynthesis and/or the remobilization of stored carbohydrates from vegetative structures. No vascular connection exists between the mother plant and the developing embryo [5, 6] so grain growth is therefore sustained by water and solute movement through cell membranes regulated by both mother plant and seed.

Seed-attached structures include coats (testa and tegmen) and other diverse maternal-originated structures, like the lemma and palea in cereals, pods in soybeans, siliques in rapeseed, and hull (ovary wall attached to the floral receptacle) in sunflower. These structures can greatly influence grain quality appreciation. The seed coat color in different types of beans (*Phaseolus*) impacts consumers differently according to the region, causing rejection of some genotypes albeit their good nutritional properties. Sorghum caryopsis with or without tannins are another example of the importance of grain coats affecting seed quality. Some seed coats can provide nutrients, like the B-group vitamins and micronutrients in cereal brans. In addition, they contribute to other important biological and technological functions, protecting the seed from mechanical damage in postharvest, or by affecting the industrial grain processing (wheat grinding, barley malting, rice parboiling). Seed coats can also impact seed dormancy and germination processes [7]. During recent years, seed-attached structures have received special attention as influencing the potential grain size and volume [8–11].

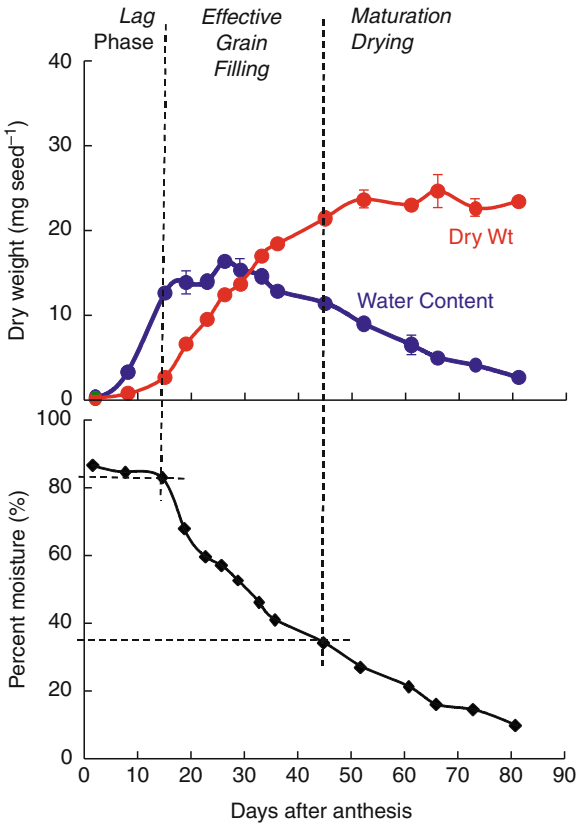
Seeds store carbohydrates (starch, oil) and proteins (soluble and insoluble). The places where these reserves are accumulated vary widely between cereals and oilseeds. In cereals, the tissue that specializes in storing starch and protein is the endosperm. In contrast, oil seeds do not have a specialized storage tissue; oil and protein accumulate in embryo and cotyledon cells. The well-developed starchy endosperm of cereals, with an outer aleurone layer, can comprise as much as 80% of the dry weight of the mature seed. The mature endosperm consists of dead cells packed with starch granules embedded in a protein matrix. The embryo is relatively small, accounting for only about 1–2% of the seed dry weight in wheat, and is usually located on one side of the seed near the point of attachment of the seed to the mother plant [3, 6]. The non-endospermic true seed of oilseeds consists of a large embryo with two cotyledons and the embryo axis. The majority of the reserve materials are stored in the cotyledons, which make up as much as 70% (sunflower) to 90% (soybean, rapeseed) of the total seed dry weight [6].

Grain structure is important since it determines grain and industrial processing quality. Cereal endosperm structure is defined by the number, shape, and size of the starch granules, together with the quantity and type of proteins in the protein matrix. Endosperm structure is used to classify wheat according to its hardness (soft, hard), thus affecting its industrial processing quality (milling capacity and flour yield). In addition, endosperm structure is used to separate dent and flint maize according to the quantity and partitioning of the floury and horny endosperm (greater proportion of horny endosperm in flint maize). Other endosperm structure characteristics that affect grain quality are vitreousness and color, both important for maize, rice, and bread and pasta wheat. Grain structure is also important for defining oilseed quality. In sunflower, the proportion of hull and embryo is an important attribute that defines oil yield, since the hull does not store oil and therefore reduces the oil concentration in the embryo. In the past 30 years, genetic improvement has reduced the hull proportion of sunflower oilseed, increasing the oil percentage on the whole seed [12]. However, thin hulls are usually harder to remove during industrial processing, so other improvement strategies are needed to increase the percentage of sunflower oil in the future. Grain structure has, therefore, a strong impact on the commercial and industrial quality of the grain, and

for this reason its attributes are present in grain marketing regulations worldwide.

Grain Growth and Source–Sink Balance

Seed Biomass During grain filling, the pollinated flower undergoes cell division and differentiation and forms a mature grain (development), which increases in size and weight (growth), reaching mature grain dry weights of 30–50 mg (wheat-barley), 250–400 mg (maize), 20–25 mg (rice), 30–50 mg (sunflower), 150–400 mg (soybeans), and 2–5 mg (rapeseed). Growth and development dynamics can be described by analyzing the rate and time period of grain growth (Fig. 2). The latter are useful tools to explain changes in the final grain weight due to genotypic and environmental factors. Species differ in their biomass per seed, and ample intra-specific differences are also observed [6].



Grain Quality in Oil and Cereal Crops. Figure 2
Dynamics of individual seed dry weight (Dry Wt), water content per seed, and seed moisture of wheat seed

Commercial genotypes used by farmers in maize, wheat, and soybean show differences in seed size, and this variability is even larger when exotic material is considered.

Seed biomass accumulation is commonly partitioned into three phases: the lag phase, the effective seed-filling period, and the maturation drying phase (Fig. 2). The lag phase is a period of active cell division. It is characterized by a rapid increase in water content with almost no dry matter accumulation. Following the lag phase, cells within the seed enter a differentiation and maturation phase, and a period of rapid dry matter accumulation resulting from the deposition of seed reserves. This phase is generally referred to as the effective seed-filling period. As in the lag phase, water content continues to increase rapidly and eventually establishes the maximum volume of the seed. Species vary considerably as to when maximum seed water content is achieved during seed filling [13]. In maize kernels, maximum water content occurs near mid seed filling [14], while in soybean seeds maximum water content is achieved at a later stage, when 70–80% of the final seed size has been achieved [15] and conversely, sunflower reaches it earlier with only 30% of final grain dry weight [10]. During the third phase of development, seeds loose water content, reach "physiological maturity" (maximum dry matter accumulation), and enter a quiescent state [3]. Seed water concentration declines throughout the three stages of seed development (Fig. 2). This decline is most obvious after seeds reach physiological maturity, but it also occurs during rapid seed filling as water is displaced by reserves [14–16].

The progress of dry matter accumulation in developing seeds and the concurrent loss of water are closely related phenomena. Studies with maize, wheat, soybean, and sunflower [17–20] have shown that final seed size is achieved at, or near, a minimum water concentration. Also, results from several studies have shown that seed water concentration accurately predicts the percent of maximum seed size achieved at any moment during seed filling in wheat, soybean, maize, and sunflower [17–20]. Such results support the notion that the duration of seed filling is determined by the interaction between reserve deposition and declining cellular water content, where deposition of reserves such as starch, protein, or lipids replace water

until a critical minimum water concentration is reached [6, 20, 21]. Species differ in the seed water concentration when they achieve maximum seed biomass [13]. For example, soybean seeds reach maturity at ~62%, maize seeds at ~36%, and wheat seeds at ~37% moisture. Although minor compared to differences across species, it has been shown that when an ample set of cultivars within a species is analyzed, variability for this trait can also be observed [22].

The rate of seed growth during the effective seed filling is highly dependent upon the number of sites for reserve deposition. The usual estimate of seed sink capacity is the number of differentiated cells during the lag phase. In maize, wheat, and other cereals, the number of endosperm cells is highly related to the rate of seed growth during rapid seed filling. In legumes such as soybean or pea, the number of cotyledon cells is highly related to the rate of seed growth. Thus, rate and duration of grain filling are important to define the final grain weight, an important attribute of grain quality.

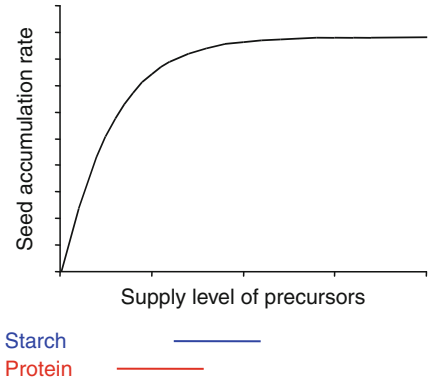
Source–Sink Balance In higher plants, nutrients from assimilation sites (sources) are delivered to sites of nutrient utilization (sinks) through an interconnected network of sieve elements. Partitioning of phloem-delivered nutrients between competing sinks is governed by their relative ability to unload major osmotic species from the importing phloem sieve elements [23]. This process depends upon a set of intercellular (post-sieve element) transport events which are integrated with growth or storage functions of the recipient sink tissues [24].

Species differ on the seed size at maturity [25], and this interspecific variability is more related the amount of assimilates available per seed during the early lag phase than during the effective seed-filling period [21]. At flowering, plants adjust the number of seeds and the potential seed size to the growth environment [21], and species differ in how they distribute available assimilates into more seeds or more potential seed size at around the period when seed number is being determined [26]. Seed size is mainly determined by the genotype, although the environment can affect the final size as well. Water availability and temperature are two environmental conditions that can create important changes in the size of the seeds at maturity.

The amount of assimilates available per seed is usually referred as the source–sink balance, and is used to describe the relation between the total amount of available assimilates and the sink number. This ratio is used to simplify the idea of assimilate availability per sink, and the way the source–sink ratio has been estimated can vary widely. Different researchers have used plant growth per seed, green leaf area per seed, sucrose availability per seed, and alternative approaches including plant growth per day per unit of sink growth per day. The source–sink balance that the seeds experience during their growth is adjusted at around flowering, when plants are setting the number of seeds.

Because plants grow in a nonuniform environmental condition, the source–sink balance during the period when seeds are accumulating biomass can change. An example can be a defoliation caused by an insect eating leaves attacking the crop at mid grain filling (which would reduce the source–sink balance of the crop) or a drought stress reducing plant growth (also reducing the source–sink balance). The source–sink balance becomes relevant because not all seed components vary to the same degree when assimilate availability per seed is altered, so the seed composition and quality may change [27–29].

Jenner and coworkers developed a theoretical model to understand how changes in the amount of assimilates available per seed can affect seed composition [28]. Their model is based on the idea that each one of the seed components can be more or less affected by changes in the level of precursors available for the growing seed because not all components are receiving from the mother plant the same level of precursors needed for their deposition within the seed. An example is illustrated in Fig. 3, where changes in the level of precursors will most surely not affect the starch content of the seed but changes in the level of precursors needed for protein synthesis will affect the protein content and the final protein concentration. This model helps explain why changes in plant growth (that affect the precursor levels) will most surely not affect starch content but will surely affect the protein content of the seed. Recent studies conducted on soybean [30] and maize compositions [27, 31] agree with this model.



Grain Quality in Oil and Cereal Crops. Figure 3
Level of precursors available per seed to synthesis of different components of seeds (Adapted from [28])

Synthesis of Major Components

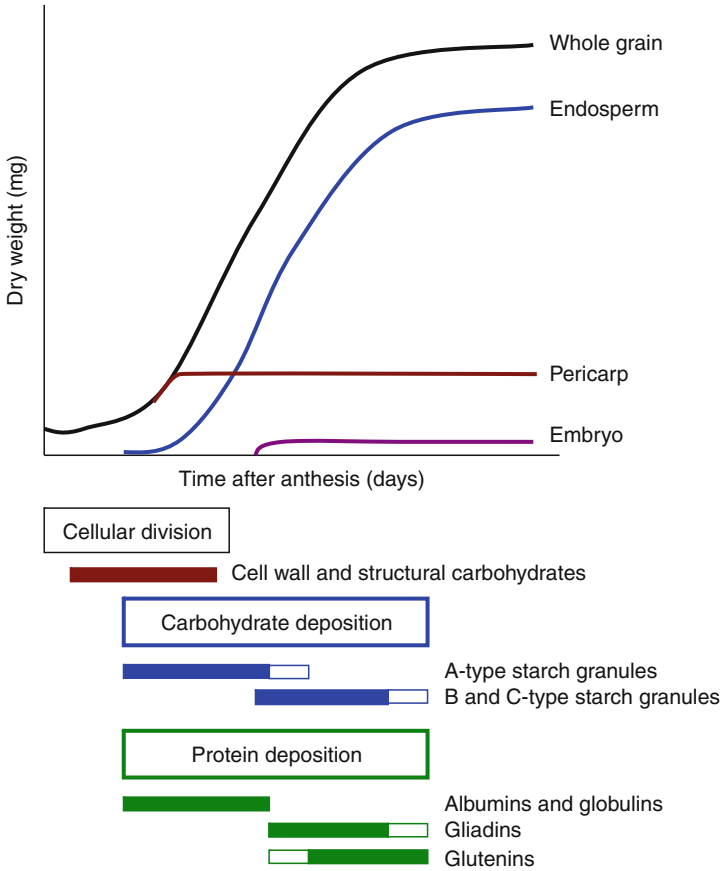
Grain growth involves growth processes of various structures (seed coats, embryo, endosperm) and accumulation of different substances (starch, oil, protein). Grain components are not synthesized simultaneously nor do they occur at the same rate; thus, physical and chemical grain composition varies during grain filling. This is an important aspect when dealing with industrial and nutritional grain quality.

As an example, the different parts of a wheat grain and the main components synthesized during grain filling are shown in Fig. 4. Starch is the main component in wheat, comprising 70–80% of the final grain weight. Different types of starch granules of varying numbers, shapes, and sizes are synthesized into the endosperm cells. The A-type granules are bigger and quantitatively more important than the succeeding B- and C-type granules. Starch is composed of amylose and amylopectin at a 3:1 ratio, except in waxy genotypes where amylopectin is more abundant.

In wheat bread, proteins comprise 5–20% of the final grain dry weight and include albumins and globulins (30–40%); gliadins and glutenins (60–70%). Albumins and globulins have enzymatic and metabolic functions and are located in the embryo and aleurone layer; gliadins and glutenins form the gluten and are reserve proteins, confined in the endosperm [32]. During grain filling, metabolic proteins are synthesized first and predominate until 10–15 days after anthesis. Reserve proteins accumulate during the effective filling phase; gliadins are the first to be detected (10–15 days after anthesis) while glutenins are deposited later (15–20 days after anthesis). Gliadins give viscosity to the mass while the glutenins confer elasticity, and both result in viscoelastic gluten appropriate for a good loaf volume. Since the gliadins:glutenins ratio changes during grain filling, crop exposure to stressful conditions (i.e., high temperature, water stress) during this phase will modify the total protein mass and the gliadins: glutenins ratio, affecting baking quality.

Grains from other species have different grain component synthesis patterns. For example, in maize there is only one type of starch granule and although starch is synthesized through the entire effective filling phase amylose accumulation occurs after amylopectin. Reserve proteins (5–14% of the final grain weight) are accumulated during the entire effective filling period as well, forming protein bodies in the endosperm cells. Oil accumulation (3–15% of the final grain weight) takes place at the end of the filling phase and most of the oil is found in the embryo. In oilseeds, oil and protein are the main reserve substances; carbohydrates are scarcely accumulated (<20%). Sunflower seeds contain 40–55% oil and 10–20% protein, while soybean seeds contain greater protein percentages (35–50%) and lower oil percentages (10–25%). Both protein and oil are deposited in the embryo cells during the linear grain filling phase. Oil deposits form oleosomes or lipid bodies of spherical shapes, while reserve proteins form dense and irregular protein bodies [33, 34].

During grain filling oilseeds, protein synthesis usually occurs after oil synthesis. Oil is formed by triglycerides, which are composed of one glycerol molecule combined with three fatty acids. Fatty acids differ in the number of carbon atoms (typically between 14 and 22 in vegetable oils) and the number of double bonds between carbon atoms. The different proportions of fatty acids modify the physicochemical and industrial properties of oils. Oils with high saturated fatty acid percentages (without double bonds; like palmitic and stearic acids) are semisolid at room temperature, with a high melting point and a higher resistance to oxidation (fat degradation due to oxygen presence). However, consumption of these oils (especially palmitic acid) increases cholesterol levels in the bloodstream. On the contrary, oils with high proportions of monounsaturated fatty acids (like oleic acid) and



Grain Quality in Oil and Cereal Crops. Figure 4
Grain filling dynamics of a cereal (wheat) and synthesis of major grain components (Adapted from [29])

polyunsaturated fatty acids (like linoleic and linolenic acids with two and three double bonds, respectively) are liquid at room temperature, have a lower melting point, and a higher susceptibility to oxidation as the number of double bonds increase; these oils are healthier than saturated fatty acids. During grain filling, the proportion of fatty acids varies according to the species and crop varieties. In traditional sunflower genotypes, the oleic:linoleic ratio decreases during grain filling while total oil accumulation increases. In contrast, in "high oleic" sunflowers, the oleic proportion is high and constant during grain filling due to the low activity of the enzyme responsible for the linoleic synthesis deriving from oleic acid [35, 36]. Oil final composition varies greatly among oilseed species, and genetic improvement has achieved a wide variety of fatty acid compositions within the same species resulting from physical and chemical mutagenesis that affect specific enzyme functions responsible for the presence of double bonds in fatty acids [37, 38]. These enzymes are also affected by the environment, producing changes in grain oil content and composition.

Main Factors Affecting Grain Quality

Genotypic Effects

Some few grain attributes are mainly driven by the genotype, and the environment has relatively low influence. For example, the color of the wheat grain (white, yellow) is strongly determined by the ability of genotype to accumulate lutein, and the character "high oleic" in oilseed genotypes is associated with genetic mutations defective for the enzyme that desaturates oleic acid. Genotypes within the same species can

Grain Quality in Oil and Cereal Crops. Table 1 Grain composition ranges reported in different species

Species	Grain composition range	Source
Soybean	33–42% protein	[35]
Sunflower	20–30% oil (confectionary type)	[33, 38]
	40–55% oil (oil type)	
Wheat bread	5–20% protein	[40]
Corn	5–14% protein	[31]
	8–12% protein (pop corn type)	[41]
	5–10% oil (high oil content)	[42]

present large differences in grain composition. For example, in commercial genotypes of soybean, the difference in protein percentage can easily vary from 33% to 42% [39]. This variability can be observed for any seed component in any species [40–42], as ample natural variation is common (Table 1).

Earlier studies working on understanding the genetic basis of genotypic differences in seed composition were based on the use of mutants, and these usually yielded qualitative differences in seed composition. For example, a commercial genotype of maize usually contains ~40% amylopectin and ~60% amylose, and a waxy mutant contains 100% amylopectin and no amylose. At present time, a large number of mutants have been discovered and used within any species as specialty quality genotypes.

The modification of the fatty acid profile of oil seeds has been one of the main tasks faced by oilseed breeders over the past 40 years. Success in this field has been of paramount importance for the worldwide expansion of some oilseed crops. The elimination of erucic acid (a harmful fatty acid) from rapeseed oil was the first step toward the development of canola (zero-erucic, low-glucosinolate rapeseed) as one of the major sources of vegetable oil in the world. Other landmarks in oilseed breeding for seed oil quality have been the development of high oleic, low linolenic acid canola, low linolenic acid linseed and soybean, high oleic acid sunflower, high saturated sunflower, and sunflower lines with modified tocopherol (antioxidant compounds) composition. Most of these traits defining seed oil quality have been found to be governed by a reduced number of genes (one to three major genes, with several alleles for each locus in most cases), and this fact implies that the practical management of single quality traits in breeding programs is relatively easy if compared with polygenic traits (as grain yield, grain weight or protein and oil content). Additionally, the fatty acid composition of the seed oil is determined by the genotype of the developing embryo (not the whole plant), so mutagenesis and selection can be carried out at a single-seed level, using the half-seed technique.

In wheat, improving yield potential without negatively affecting grain quality is difficult, mainly because increases in grain yield are generally accompanied by a decrease in grain protein content, which is strongly associated with bread-making quality. Wheat breeders give grain quality the same level of importance as yield potential and disease resistance. In contrast to the low heritability of protein content, grain hardness and yellow pigment are highly heritable and can be readily improved through conventional breeding. Plant breeders select at least one parent with the desired quality when designing their crossing strategies, particularly as end-use requirements frequently determine the fate of potential new cultivars, but the stage in the breeding process at which quality determination takes place will influence which tests (micro or macro tests) are applied, according to the sample size available.

At present, natural variation for seed composition is being studied identifying quantitative trait loci (QTLs) for different seed components (oil, protein), as any seed component is a quantitative trait governed by many genes and each one with an individual small effect. The study by Blanco and coworkers can be mentioned as an example, where the authors studied seed protein concentration in wheat where three major QTLs were detected [43]. This methodology is currently becoming very popular and has yielded molecular markers associated with seed component traits that can help understand the genetic bases of the trait and be used by breeders and the industry.

Environmental Effects

As mentioned earlier, the majority of quality traits are greatly modified by the environment and by genotype–environment interactions. Grain weight and protein concentration are found within this group of traits.

Environment variables like high temperature, water and nitrogen (N) availabilities have been the most studied modifying grain quality.

The response in grain composition to a particular stress depends mainly on the stress characteristics (i.e., intensity, duration of the stressful period, opportunity of occurrence, and the interaction that this stress may have with other stresses to which the crop is exposed to). The relevance of the intensity and duration of the stress on the magnitude of the change in grain composition is self-explained (the more severe and the longer a stress is, the greater change in composition produced, though not necessarily this implies that the relationship is linear). The timing of occurrence is also critical, as shown in Fig. 4 not all stages are equally critical for the final determination of grain quality: if the stress coincides with critical stages for synthesis and deposition of the components, the changes will result far stronger than that of stresses occurring is less-critical stages. Therefore, the grain composition responses to stressful factors may range from virtual insensitivity (if punctual synthesis reductions are compensated by recovering when no stress occurs) to different ranges of quality reductions to even crop failure to produce a certain quality level.

Seed growth and development are responsive to temperature, but their responses vary with the temperature range considered [44]. As a general rule, the rate of seed development increases as temperature increases, reducing the duration of seed-filling period. At lower temperatures, seed growth rates decrease linearly as temperatures fall below 15°C in wheat, soybean, rice, sunflower, and maize. Seed growth rates increase when temperatures rise from 20°C to 30°C; however, this increase does not offset the linear decrease in seed-filling duration, resulting in lower grain weights [44]. In most cases, moderately high temperatures (20–30°C) prevail during grain filling, although short periods of very high temperatures (>30–32°C) may occur reducing seed growth rate and causing the early end of grain filling period. In addition, the earlier the heat stress, the greater the impact on grain weight [45, 46]. Brief periods of high temperatures can cause reductions in grain weight, but these effects can be overlooked if only the average temperature during post-flowering period is considered. Thus, moderately high temperatures (20–30°C) during the post-flowering period reduced grain weight mainly through shortening the grain filling period, while very high temperatures (>30–32°C) even for a few days can reduce grain weight by reducing grain filling rate and the early cessation of grain growth period. Both aspects of post-flowering temperature should be considered especially because climatic change could bring about high-temperature scenarios in the next decades, together with an increase in heat-stress events [47, 48].

Grain quality and composition are also affected by temperature. Several experiments suggest that the temperature effects on seed composition are related to dry matter metabolism and accumulation. The timing, intensity, and duration of occurrence of heat stress may alter final grain quality according to the grain component synthesis process involved (carbohydrates, proteins, oils). Interestingly, there are some reports on the possibility of recovery post-stress [49, 50]. In wheat and barley, protein percentage increases with increasing temperatures (15–30°C) because the negative impact of high temperatures on starch synthesis is greater than the impact on protein synthesis, thus decreasing the starch proportion in the grains [28]. High temperatures also affect protein quality, generally increasing gliadin:glutenin ratio, which causes weak dough with a low bread-making quality. The temperature impact on wheat grain quality will therefore depend on the balance between the positive (higher protein) and negative (greater gliadin:glutenin ratio) effects. Temperature also affects oil fatty acid composition in oilseeds [51]. The higher the temperatures during grain filling, the higher the fatty acid saturation (i.e., greater proportions of oleic acids and lower proportions of linoleic and linolenic acids) due to the reduced activity of unsaturation enzymes in grains [52]. Temperatures registered during the night in early grain filling phases have shown to have the best predictive values for modeling the final oil composition in sunflower [53]. Progress in modeling the quality of other grains is underway [54].

In field crops, high-temperature occurrences are commonly associated to water stress, increasing the negative temperature effects. Drought stress produces a shortage of assimilates and often reduced N availability, which cause a reduction in grain growth. In general, a drought episode occurring after flowering

has a similar effect as an increase in temperature – the quantity (mg grain^{-1}) of protein per grain remains stable, while starch accumulation in grain is significantly reduced, resulting in smaller grains with a greater protein percentage [55]. In oilseeds, post-flowering droughts decrease grain oil percentages and increase protein percentages [56, 57] indicating that carbon metabolism is affected to a greater extent than N metabolism. Water stress has a smaller impact on fatty acid composition; in general, droughts do not modify the saturation degree in oils except under severe stress conditions which produce an early grain-filling cessation [58].

N availability also affects final cereal and oilseed grain composition. In general, when soil N availability is low, cereal crop yields respond positively to N fertilization. A dilution effect occurs when N taken up by the crop is partitioned in a greater number of grains, which reduces grain protein percentage. If N availability is further increased, both crop yield and grain protein percentage are increased. In addition, the stage of development when N is added is important in defining wheat grain quality. N applications around flowering increase nitrogen availability per grain, increasing protein percentage. It is reported that increases in N availability result in increases of gliadin:glutenin ratio, which in turn produce a weakening of the dough [59]. In oilseeds, a greater soil N availability increases crop yield and grain protein percentage. Consequently, oil percentages in grain decrease due to the negative relationship between oil and protein (expressed as a percentage of the grain weight). Nitrogen application effects on the grain fatty acid composition are smaller and more variable compared to temperature and water stress effects [60, 61]. A greater knowledge on the physiological processes that regulate the responses to these environmental factors is essential to decide the management of the crop to produce grain for a specific end use.

Management Strategies

Although both grain yield and quality are determined throughout the growing season, important decisions that will strongly affect them should be taken before planting [62]. The farmer's choice of genotype and the amount of nitrogen available are central for successfully combining the genotype potential for yield and quality with the environmental availability of resources. As stated earlier, final grain quality is the result of the interaction between the genotype, the natural environment, and the crop management practices [63]. In extensive production systems, it is not possible to provide each stage of the crop cycle with the optimal combination of environmental factors to reach the highest possible yield and quality, therefore, a trade-off is to make preplanting decisions to ensure that critical crop stages for the definition of yield and quality are given a preferential environment [62]. Nevertheless, knowledge of the effects of environment and G × E interaction is still rather imprecise, so management strategies with the objective of increasing yields, while obtaining high quality, are difficult to design.

There are a number of grain quality attributes that are strongly governed by the genotype and therefore choosing the proper genotype in relation to the final end use of the grain is critical. In several countries, for trading purposes wheat is classified into distinct categories of endosperm hardness (soft, semihard, and hard). Grain hardness is determined by the packing of grain components in the endosperm cells [40] and according to this attribute, the end product can vary from pasta (hard endosperm), biscuits (soft endosperm), to bread (hard endosperm). Usually, this classification can be more detailed and complex [64]. In the case of sunflower, oil fatty acid composition is genetically controlled [65], and the oil composition has been modified mostly by altering the function of major genes through mutagenesis [38].

Addition of nitrogen fertilizer is one of the most frequent management practices for altering grain quality (and of course grain yield). It is difficult a priori to know the effect of adding nitrogen to grain quality as many other factors are intervening and modify the final expected result. In the case of wheat crops, the initial amount of nitrogen in the soil, the specific moment of fertilization, the amount of available water, and rain pattern during the growth cycle as well as plant density at sowing and genotype nitrogen use efficiency are the main factors that interact and may modify the final response in grain quality. In general, it is accepted that regardless of the species, the increase in grain yield leads to a decrease in the protein to starch or oil ratio. This negative relationship between yield and grain

N concentration reflects the fact that carbon assimilation and accumulation during the grain filling period is sink-limited [66] while nitrogen accumulation in grains is usually source-limited [67], as a result of dilution effects. The final protein concentration will thus depend on the balance between the source capacity to provide nitrogen and the strength of the sink for accumulating carbohydrates [68].

Future Directions

The compositional requirements for a particular grain vary from one product to the other depending on its end use. In addition, grain quality is a dynamic concept as it changes constantly as new uses can be developed for particular grains. The three major pillars of grain composition are: the genotypes, the environments during grain growth, and their interaction.

On the genetic pillar, the knowledge gained in the recent past has been extraordinary. Based on the molecular tools developed, a number of genes and QTLs involved in the determination of particular grain components (in turn determining grain quality attributes) have been identified and mapped in several crops, and it seems easy to predict that in the near future almost any breeding program in the world will be able to manipulate these genetic factors with certainty.

Regarding the environment during grain filling, important and useful findings have been reported in relation to high temperatures, and in lesser extent in water stress, and nitrogen availability. Few studies have attempted to examine the interactions between these environmental factors on grain quality attributes. It has been recently reported that high-temperature stress effects may be mitigated under high nitrogen availability for wheat and barley [69–71].

Undoubtedly, the challenge for breeders and agronomist is dealing with G × E interactions [72]. Therefore, there is a need for increasing current knowledge on the physiology of quality traits in order to obtain both high yield and high quality through breeding and management strategies. This will also help predict grain composition through a series of genotypes and environments.

Using agronomic simulation models properly calibrated and validated for the target population of environments can be a tool for understanding and predict final grain composition. The incorporation of grain quality modules into crop simulation models is increasing (European Journal of Agronomy 25, 2006). Grain protein content was the first trait incorporated into modeling as well as grain size (grain weight) which is a quality criteria especially valued by millers in the case of cereals but also for oil extraction in oil crops. Recently, more detailed concepts have been incorporated such as the type of protein [73] and oil quality [74]. It is expected that incorporating genetic data into simulation routines will be done in the near future.

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