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Review

Transgenic plants for insect resistance

Lise Jouanin [a,*], Michel Bonadé-Bottino [a], Cécile Girard [a], Gil Morrot [a], Marc Giband [a,b]

[a] *Biologie Cellulaire, INRA, Route de Saint Cyr, 78026 Versailles Cedex, France*
[b] *CIRAD-CA, Biologie Cellulaire, INRA, Route de Saint Cyr, 78026 Versailles Cedex, France*

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Abstract

Plant genetic engineering offers opportunities for the creation of insect-resistant plants by insertion and expression in planta of entomopathogenic proteins. Two main approaches to obtain such plants have been explored. The first one involves the use of delta-endotoxin coding sequences originating from the bacterium *Bacillus thuringiensis*. The second approach uses plant-derived genes, such as those encoding enzyme inhibitors or lectins. Much work throughout the world is devoted to obtaining plants of different species expressing such genes and showing resistance to insect pests. Research projects under development, and an assessment of the situation and of the problems encountered on the way to a commercial use of such transgenic plants are discussed in this review. © 1998 Elsevier Science Ireland Ltd.

Keywords: Transgenic plants; Insecticidal proteins; *B. thuringiensis*; Enzyme inhibitors; Lectins; Resistance management

1. Introduction

Losses due to pests and diseases have been estimated at 37% of the agricultural production world-wide, with 13% due to insects [1]. Present methods of crop protection rely mainly on the use of agrochemicals. For the future, it is necessary to develop a more environmentally friendly agriculture which will have decreased inputs in energy and chemicals, and will not generate harmful outputs such as pesticide residues. With this aim in view, the resistance of plants to pests and pathogens must be improved. Some success has been achieved towards this aim using conventional plant breeding techniques and in vitro techniques.

* Corresponding author. Tel.: + 33 1 30833063; fax: + 33 1 30833099; e-mail: jouanin@versailles.inra.fr

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For example, somatic hybrid potato lines resistant to the Colorado potato beetle have been obtained by electrofusion of protoplasts isolated from a wild potato species, *Solanum chacoense*, and from a cultivated one (*S. tuberosum*) [2]. The new technology of plant genetic engineering offers the possibility of introducing resistance genes from foreign species into crop plants. Different approaches to obtain insect-resistant plants are presently being explored. The main approach uses δ-endotoxin coding sequences originating from the bacterium *Bacillus thuringiensis* and the culmination of this process occurred in 1996 when the first generation of insecticidal plants were introduced on the market. Other strategies use plant-derived genes, such as those encoding enzyme inhibitors or lectins. Novel insecticidal genes are being sought or are under study in order to broaden the spectrum of this strategy and include important pests that are not sensitive to toxins already used. In this paper, our purpose is to review the work in progress in this field.

2. Use of *Bacillus thuringiensis* δ-endotoxins

2.1. The B. thuringiensis toxins

The bacterium *B. thuringiensis* was first discovered in Japan in 1902 in a silkworm rearing unit. In 1911, it was again isolated in a flour moth population and characterized by Berliner in Thüringen (Germany). *B. thuringiensis* is a gram positive bacterium that synthetizes insecticidal cristalline inclusions during sporulation. The crystalline structure of the inclusion is made up of protoxin subunits, called δ-endotoxins. Most *B. thuringiensis* strains produce several crystalline proteins (Cry proteins), each of which shows a rather narrow host range [3].

At least 90 genes encoding protoxins from a wide range of *B. thuringiensis* isolates have been isolated and sequenced [4]. The genes were first classified in different classes *cry*I, *cry*II, *cry*III [5] based on protein structural homologies and host range. CryI toxins are active against Lepidoptera while CryIII are active against Coleoptera. More recent analysis of the nucleotide sequence reveals that this classification is not necessarily based on homology or evolutionary relationships, and a new nomenclature has been proposed [6,7].

The δ-endotoxins are solubilized in the insect midgut and are activated by gut proteases that cleave the protein into a smaller polypeptide, the toxin. This toxin binds to the surface of epithelial cells in the midgut, inducing lesions that destroy the cells and lead to the death of the insect [8].

B. thuringiensis was first used as a bioinsecticide and the main advantage of such formulations is that they are harmless to humans, mammals and to the non-target fauna. Of the bioinsecticides in use, 90% are based on *B. thuringiensis*, representing in 1992 2% of the global world pesticide market [9]. However, due to their low field persistance, the use of *B. thuringiensis* sprays is still relatively limited.

2.2. Transgenic plants

The approach consisting in the transfer and expression of *B. thuringiensis* toxin-encoding genes into plants has attracted much attention. Indeed, such a system allows the entire plant to be protected, especially against insects such as borers that infect plant parts that sprays often cannot reach. Furthermore, the toxin affects the more susceptible early instar stages of the insect and the system is environmentally safe because the product is retained within the plant tissues.

The first results concerning the transfer of *B. thuringiensis* genes in tobacco and tomato were published in 1987 [10–12]. Since then, *B. thuringiensis* genes have been transferred to a number of other crop species such as cotton, rice, maize..., with Lepidoptera as the main targets. Table 1 illustrates some of the results that have been obtained. This table does not extensively review the work published in this field since a very recent review is completely devoted to this strategy [4].

The first publications reported a very low level of expression (generally less than 0.001% of leaf soluble proteins) of the *cry* genes in comparison to other genes transferred to plants. In contrast to most plant genes, these bacterial genes are extremely rich in A + T nucleotides. It has been

Table 1
Some examples of insect-resistant transgenic plants that have been obtained through the transfer of *B. thuringiensis* toxin genes

Plant	Gene	Type	Target insect	Reference
Tobacco	*cryIA(a)*	WT	*Manduca sexta* (L)	Barton et al. [11]
	cryIA(b)	WT	*Manduca sexta* (L)	Vaeck et al. [10]
	cryIA(b)&(c)	PM	*Manduca sexta* (L)	Perlak et al. [14]
	cryIA(b)	WT	*Manduca sexta* (L)	Willians et al. [24]
(Chloroplasts)	*cryIA(c)*	WT	*H. virescens*, *H. zea*, *S. exigua* (L)	McBride et al. [25]
	cryIC	S	*Spodoptera littoralis (L)*	Strizhov et al. [22]
Tomato	*cryIA(b)*	WT	*Heliothis virescens* (L)	Fischoff et al. [12]
Cotton	*cryIA(b)&(c)*	S	*H. zea*, *H. virescens* (L)	Perlak et al. [13]
Potato	*cryIA(b)*	WT	*Phthorimaea operculella* (L)	Peferoen et al. [73]
	cryIIIA	S	*Leptinotarsa decemlineata* (C)	Adang et al. [16]
	cryIIIA	S	*Leptinotarsa decemlineata* (C)	Perlak et al. [15]
Alfalfa	*cryIC*	S	*Spodoptera littoralis (L)*	Strizhov et al. [22]
Canola	*cryIA(c)*	S	*Plutella xylostella*, *H. zea* (L) *Trichoplusia ni*, *S. exigua (*L)	Stewart et al. [21]
Soybean	*cryIA(c)*	S	*H. zea*, *H. virescens* (L) *Pseudoplusia includens* (L)	Stewart et al. [74]
Maize	*cryIA(b)*	S	*Ostrinia nubilabis (L)*	Koziel et al. [19]
Rice	*cryIA(b)*	S	*Chilo suppressalis* (L) and *Cnaphalocrosis medinalis* (L)	Fujimoto et al. [18] Wünn et al. [23]
Poplar	*cryIA(a)*	PM	*Lymantria dispar* (L)	McCown et al. [17]
	cryIIIA	WT	*Chrysomela tremulae* (C)	Cornu et al. [75]

L, Lepidoptera; C, Coleoptera; WT, native gene; PM, partly modified; S, synthetic gene.

speculated that the codon usage of the *cry* genes is far from optimal for expression in plants, that polyadenylation signals within the coding region, as well as other features reduce transcript stability. Partially or totally synthetic genes were constructed ([13–23], for a review see [4]). In these genes, the nucleotide sequence is modified without changing the amino acid sequence. In plants expressing such synthetic genes, the level of expression of proteins is higher (0.02–1% of leaf soluble proteins) than with those expressing bacterial *cry* genes. Field trials have been initiated with the two types of genes, and more convincing results were obtained with the synthetic genes. Several partially or totally synthetic genes have been constructed, most of them active against Lepidoptera (*cryIA(a)* [17], *cryIA(b)* [13,18–20], *cryIA(c)* [13,20,21], *cryIC* [22,23]) while others are active against Coleoptera such as the Colorado potato beetle (*cryIIIA*) [15,16]. The codon usage can be optimized for expression in dicots or in monocots [19]. In the latter case, gene expression was also increased by the insertion of introns in the 5′ untranslated sequence.

In most cases, the *B. thuringiensis* toxin coding sequences have been placed under the control of constitutive promoters (CaMV 35S promoter or derivatives thereof). However, in some cases, tissue-specific promoters (a maize PEPC promoter expressed in green tissues and a maize pollen specific promoter) [19] or wound-inducible promoters (the pathogenesis-related PR-1a gene [24] or the *A. tumefaciens* mannopine synthase [10]) were also used.

Partial or complete resynthesis of *B. thuringiensis* toxin genes is cumbersome; recently, McBride et al. [25] integrated a native *B. thuringiensis* gene into the chloroplast genome of tobacco by homologous recombination. Very high levels of expression of the native gene were obtained, and toxin production reached 3–5% of the leaf soluble proteins. Nevertheless, choroplast transformation is far from being routinely achieved, and is until now restricted to tobacco.

The first field trials with transgenic crops expressing *B. thuringiensis* toxins were conducted in 1986 with tobacco, and several small-scale then

large-scale field tests were performed in the US and Europe (listed in [26]). In 1995, the first transgenic plants, corn expressing the CryIA(b) toxin (Maximizer™ from Novartis), cotton expressing the CryIA(c) toxin (Bollgard™ from Monsanto) and potato expressing the CryIIIA toxin (Newleaf™ from Monsanto) were approved for sale in the US. In 1996, *B. thuringiensis* transgenic crops occupied over 1.2 million ha in this country. Most of these varieties are also commercialized in other countries such as Argentina and Australia. Until now, the commercial deployment of transgenic crops has not been approved in Europe.

Screening of new strains of *B. thuringiensis* has allowed the discovery of genes with new specificities [27]. So far, new *B. thuringiensis* toxins have mostly been found through bioassay screening. More sophisticated screening methods (antibody, PCR) are underway to try to identify toxins with new insect insecticidal spectra.

3. Use of plant-derived genes

The synthesis of antimetabolic proteins, which interfere with the digestive processes in insects, is a defence strategy that plants use extensively. Such proteins can be synthetized constitutively, in tissues that are particularly vulnerable to attacks such as seeds, or can be induced by mechanical wounding, as it is the case when chewing insects feed on leaves. In many cases, evidence exists for the defensive role of enzyme inhibitors in protecting plants against insect pests [28]. Protease inhibitors purified from different plant sources have shown deleterious effects in in vivo artificial diet bioassays and in in vitro assays with insect gut proteases (reviewed in [1]and [29]). These molecules interfere with the growth and development of the larvae and in some cases cause the death of the insect.

3.1. Proteinase inhibitors

According to their specificity, proteinase inhibitors (PIs) can be divided in four classes, inhibiting serine, cysteine, metallo- or aspartyl-proteases. Plant PIs are small proteins and most of the serine PIs possess two active sites which inhibit trypsin and chymotrypsin. Serine and cysteine PIs are abundant in seeds and storage tissues of plants (reviewed in [30]). In some cases, they can be induced by wounding and insect attack [31,32]. Diet incorporation assays or in vitro inhibition of digestive proteases studies have demonstrated the potential of PIs to interfere with insect larval growth [29,33]. In addition, the expression of an antisense prosystemin gene in transgenic tomato decreased the ability of the plants to produce PIs and consequently reduced resistance towards *Manduca sexta* larvae [34].

The first gene to be successfully transferred to another plant species resulting in enhanced insect resistance was isolated from cowpea and encoded the trypsin/trypsin inhibitor CpTI [35]. The protein is an effective antimetabolite against a range of field and storage pests [1]. Lepidoptera and Diptera possess mainly serine proteinases and the obtention of plants more resistant to Lepidoptera through the use of serine PIs of different origins has been reported (Table 2).

Fewer cysteine PIs are known, and their action on insects has been less studied. Studies of the protease content of the guts of different Coleoptera have shown the presence of cysteine proteases, which constitute sometimes the major type [36]. The cDNA of a gene encoding a cysteine PI isolated from rice [37] was introduced into different plants (tobacco, potato, poplar, oilseed rape, cotton) but only results reporting the toxicity of such plants against a beetle feeding on poplar have been published [38].

Mounting evidence shows that insects can adapt to the ingestion of PIs (reviewed in [39]). Lepidoptera and Coleoptera can overexpress existing gut proteases, or induce the production of new types that are insensitive to the introduced PIs and overcome the deleterious effect of PI ingestion. Insects possess a complex pool of proteases and to avoid adaptation, the major proteases must be inhibited. This could be achieved by expressing PIs of different types, and/or by improving the affinity of the introduced PI for the target insect proteases [40]. The latter strategy can be tested using engineered PIs with

Table 2
Examples of plants with enhanced resistance towards insects by expression of enzyme inhibitors

Plant	Gene	Type	Target insect	Reference
Tobacco	CpTI	Cowpea serine PI	*Manduca sexta* (L)	Hilder et al., 1987 [35]
			Heliothis virescens (L)	Gatehouse et al., 1992 [1]
Tobacco	PPI-II	Potato serine PI	*Manduca sexta* (L)	Johnson et al., 1989 [76]
	TI-II	Tomato serine PI	*Manduca sexta* (L)	
Tobacco	PPI-II	Potato serine PI	*Chrysodeixis eriosoma* (L)	McManus et al., 1994 [77]
Tobacco	spTi-1	Sweet potato PI	*Spodoptera litura* (L)	Yeh et al., 1997 [78]
Rice	CpTI	Cowpea serine PI	*Seramia inferens* (L)	Duan et al., 1996 [79]
Rice	PPI-II	Potato serine PI	*Seramia inferens* (L)	Xu et al., 1996 [80]
			Chilo suppressalis (L)	
Potato	CpTI	Cowpea PI	*Lacanobia oleracea* (L)	Gatehouse et al., 1997 [50]
Poplar	OCI	Rice cysteine PI	*Chrysomela tremulae* (C)	Leplé et al., 1995 [38]
Pea	α-AI	Bean α-amylase I	*Callosobruchus maculatus* (C)	Shade et al., 1994 [44]
Pea	α-AI	Bean α-amylase I	*Bruchus pisorum* (C)	Schroeder et al., 1995 [45]
Azuki bean	*α-AI*	Bean α-amylase I	*Callosobruchus chinensis*(C)	Ishimoto et al., 1996 [46]
			C. maculatus, *C. analis* (C)	

L, Lepidoptera; C, Coleoptera.

affinities optimized for proteases either by computer modelling, as already performed by Urwin et al. [41] or by phage display [42] as proposed by Jonsgma et al. [43]. The combination of PIs inhibiting different types of proteases or the association of PIs with other resistance gene is a strategy worth exploring.

3.2. α-Amylase inhibitors

The commun bean, *Phaseolus vulgaris*, contains a family of related seed proteins (PHA-E and -L, arcelin, and α-amylase (α-AI)). PHA-E and -L are classical lectins with strong agglutinin activity, but α-AI forms a complex with certain amylases and is supposed to play a role in plant defense against insects. The introduction and expression of the bean α-AI gene under the control of the 5′ and 3′ regions of the bean phytohemagglutinin gene in pea confers resistance to the bruchid beetles, *Callosobruchus maculatus* and *C. chinensis* [44]. In addition to these pests of stored grain, Schroeder et al. [45] demonstrated that this gene is also able to confer resistance to another bruchid beetle, *Bruchus pisorum*, which attacks crops growing in the field. The transfer of this α-AI gene to Azuki bean confered resistance to three species of bruchids [46] (Table 2). However, in nature, *Acanthoscelides obtectus* and *Zabrotes subfasciatus*, two other bruchids, can feed on plants producing α-AI because they possess a serine protease able to cleave some kinds of α-AI [47]. It is therefore difficult to evaluate the long term interest of the expression of these genes in plants.

3.3. Lectins

Lectins are carbohydrate-binding proteins found in many plant tissues, and are abundant in the seeds and storage tissues of some plant species. The toxicity of this type of protein to mammals and birds is well documented. The toxicity of different lectins towards insects has been observed [48], however the exact mechanism of action is not clear. It likely involves the specific binding of the lectin to glycoconjugates located in the midgut of the insect, but several possible interactions could occur [49]. Most of the plant lectins, such as the wheat germ agglutinin (WGA) present antinutrient properties in animal food. Lectins such as those purified from snowdrop or garlic are toxic to insects but not to mammals [33]. Tobacco plants expressing a pea lectin were shown to be toxic to the Lepidoptera *Heliothis virescens* [1], and potato plants expressing the snowdrop lectin (GNA) were toxic to the Lepidoptera *Lacanobia*

Table 3
Examples of plants with enhanced resistance toward insects by expression of genes of different origins

Plant	Protein	Type	Insect	Reference
N. plumbaginofolia	IPT	Cytokinin synthesis	*Manduca sexta*	Smigocky et al., 1993 [59]
Alfalfa	*M. sexta* PI	Insect serine PI	*Frankliniella* spp (thrips)	Thomas et al., 1994 [57]
Tobacco	*M. sexta* PI	Insect serine PI	*Bemisia tabaci* (D)	Thomas et al., 1995 [56]
Tobacco	GNA	Snowdrop lectin	*Myzus persicae* (A)	Hilder et al., 1995 [51]
			Aulacorthum solani (A)	Down et al., 1997 [52]
Potato	GNA	Snowdrop lectin	*Lacanobia oleracea* (L)	Gatehouse et al., 1997 [50]
			Myzus persicae (A)	Gatehouse et al., 1996 [53]
Potato	BCH	Bean chitinase	*Lacanobia oleracea* (L)	Gatehouse et al., 1997 [50]

(L), Lepidoptera; (C), Coleoptera; (A), Aphids; (D), Diptera.

oleracea [50]. Most work involving lectins has focused on the obtention of aphid-resistant plants. The expression of the GNA in tobacco resulted in added protection against the aphid *Myzus persicae* [51], and in potato against the aphids *M. persicae* and *Aulacorthum solani* [52,53] (Table 3). To date, no evaluation of the long term interest of this strategy has been published.

3.4. Chitinases, tryptophan decarboxylase

Insects contain chitin, not only as an exoskeletal material, but also at the level of the peritrophic membrane. Chitinase activity could therefore interfere with digestion. The expression of a bean chitinase in potato causes no deleterious effect to a Lepidoptera, *Lacanobia oleracea*, but reduces fecondity of the aphid *A. solani* [50]. However, the expression of a chitinase of insect origin in transgenic plants seemed to be more effective in causing larval mortality to a beetle, *Oryzaephilis mercator* (see next paragraph).

Alkaloids are often considered as antifeedant for many insects. The expression of the tryptophan decarboxylase gene from *Catharanthus roseus* allows the synthesis of tryptamine and tryptamine-based alkaloids in tobacco, a plant which originally does not show thryptophan decarboxylase activity. The production of tryptamine in transgenic tobacco causes a decrease in whitefly (*Bemisia tabaci*) pupae emergence [54]. The mechanism by which tryptamine may interfere with insects is unknown.

Other antinutritive and toxic compounds are supposed to be involved in plant defense against insects (reviewed in [55]). Among them, tomatine, polyphenol peroxidase (PPO) and lipoxygenase (LOX) have been shown to be toxic to insects but until now, no report has shown that their overexpression in plants confers higher resistance against insects.

3.5. Genes of other origin

Proteinase inhibitors are present in different kingdoms and their activity spectrum could be different from that of plant origin. The expression of the insect *Manduca sexta* PI in tobacco was effective against *Bemisia tabacci*, a whitefly [56] and against thrips (*Frankliniella* spp.) predation in alfalfa [57].

A recombinant *Manduca sexta* insect chitinase purified from transgenic tobacco plants was toxic for the merchant grain beetle *Oryzaphilus mercator* when administered orally in the diet at a level of 2% [58]. These data indicate that insect chitinases are a potential factor of resistance and might be more potent than chitinases from other sources.

The bacterial isopentenyl transferase (*ipt*) gene is involved in cytokinin biosynthesis. Its expression in plants is not compatible with the obtention of transgenic plants with a normal phenotype. Smigocki et al. [59] introduced this gene in *Nicotiana plumbaginifolia* under the control of the wound-inducible promoter of the potato proteinase inhibitor II gene. The expression of

this gene (or of genes involved in the production of secondary metabolites induced by the expression of the *ipt* gene) is deleterious to *Manduca sexta* (a Lepidoptera) and to *Myzus persicae* (an aphid) [59]. The mechanism involved is not clear, but the role of secondary metabolites is strongly suspected.

3.6. New insecticidal genes

Many studies are under way in order to identify new insecticidal products. One strategy is to screen new sources for potential insecticidal proteins in a random fashion. Sources for screening include plant samples, particulary tropical plants with well-known insecticidal properties, and bacteria at different physiological stages. Results in this latter field of research are particularly interesting. During their vegetative growth, some *Bacillus* species become the source of insecticidal activities: *B. thuringiensis* produces a protein, Vip3A, active against lepidopteran insects such as the black cutworn (*Agrotis ipsilon*), a corn pest [60]. *Streptomyces* cultures secrete a cholesterol oxydase active against the boll weevil (*Anthonomus grandis*) [61,62]. These proteins are highly toxic (within the same range as *B. thuringiensis* toxins) and could be new interesting sources for engineering resistance. Because of the bacterial origin of these genes, synthetic genes may have to be constructed to reach high enough levels of expression, as it is the case for *B. thuringiensis* genes.

4. Management strategies for insect-resistant plants

Engineering crops with insecticidal protein genes is one of first major projects in plant biotechnology. The value of such technology to the seed/biotechnology industry, the farmer, the environment and the consumer is obvious. Insect-resistant crops could reduce the cost, time and efforts spent protecting crops from insects and could contribute to an environmentally friendly production system. However, transgenic plants need to be integrated in pest management strategies. Insects have demonstrated a high capacity to develop resistance to a wide array of chemical insecticides [63]. Recently, with *B. thuringiensis*-based insecticides, resistance has developped in field populations of *Plutella xylostella* [64] and different insects have developed resistance to *B. thuringiensis* toxins in laboratory conditions (reviewed in [65]). Therefore, one of the most critical aspects of the use of insect-resistant transgenic crops will be their safe deployment. If the protection afforded is to be durable, these crops require management strategies similar to those implemented for crops harbouring resistance genes introduced though traditionnal breeding. At present, these management strategies concern *B. thuringiensis*-expressing plants since they have already reached the market. Many of the alleles confering insect resistance are recessive, and management strategies have focused on ensuring that a sufficient number of susceptible insects are present, thus ensuring that the resistance allele will not be fixed in the population. Strategies to retain susceptibility to *B. thuringiensis* genes have already been proposed [66] and are discussed in different publications [26,27,67]. These strategies (Table 4) are based on existing methods for the management of insecticide resistance, and are not mutually exclusive.

Table 4
Tactics available for deploying plants harbouring insecticidal genes (source [66])

Gene strategies	Single gene
	Multiple genes (e.g. pyramid)
	Chimeric genes
Gene promoter	Constitutive
	Tissue-specific
	Inducible (e.g. wounding)
Gene expression	High dose
	Low dose
	Mixtures
Field tactics	Uniform single gene
	Mixture of genes
	Gene rotation
	Mosaic planting
	Refuges (spatial, temporal)

Most of the existing insect-resistant plants express a single resistance gene placed under the control of a constitutive promoter, and management practices were first adopted in the US for the deployment of such *B. thuringiensis* toxin-expressing plants. The prefered method of resistance management is refered to as the 'high dose/refuge' strategy [27] which aims at eliminating (or diluting) the resistance allele and thus preventing it from becoming widespread in the insect population. This involves the use of plants expressing the selected toxin in the tissues that are attacked by pests at a level high enough to kill heterozygous (or even homozygous) resistant insects [68]. Associated with such toxin-expressing plants, untransformed plants (or refuges) on which sensitive insects could multiply, would allow the 'dilution' of the resistant allele through random mating of sensitive and resistant insects. These plants may be supplied as pure stands (untreated or treated with conventional insecticides) alongside the transgenic plants, or as in-field refuges (mosaics). Pure stands of non transgenic plants are the prefered option, due to the difficulty in implementing mosaics.

The deployment of multiple resistance genes or gene pyramiding, requires the incorporation in the plant genome of genes encoding two or more toxins possessing different modes of action. This could be achieved though the use of several *B. thuringiensis* genes, after demonstration that the target receptors of the Cry proteins are different. Laboratory studies will be necessary to ensure that no cross resistance occurs, as already observed by Tabashnik [69]. The use of a *B. thuringiensis* toxin gene in association with other resistance genes (PI, lectins, Vip) could constitute a better approach [70].

The constitutive expression of the toxin in transgenic plants may cause a high pressure of selection on insect populations. Specific promoters could be used, thereby limiting insect exposure to the toxin which could be expressed in certain parts of the plant attacked by the insect (tissue-specific), at the most sensitive growth stages (temporal-specific) or in response to insect feeding (wound-specific). This targeted expression of the insecticidal gene could also ensure public acceptance of transgenic plants. For example, toxin does not need to be expressed in potato tubers but only in leaves to control the Colorado potato beetle. Progress in the understanding of gene regulation will be very useful in developing targeted expression.

However, the deployment of transgenic crops is still very recent, and guidelines are mainly based on theoretical models. It is clear that for each crop, pest and area, there will be different requirements. The high dose/refuge strategy requires a dedicated effort from the biotechnology companies and from the farmers. It should be considered that we are still at the experimental stage of the integration of transgenic plants into traditional farming systems. The future of these plants depends on their rational use and on the development of proper guidelines.

5. Prospects

In nature, many strategies are used by plants to protect themselves against insects [55]. Various secondary metabolites are toxic to insects, but their biosynthetic pathways are rarely known or too complex for them to be used to create insect-resistant plants [71]. This fact explains the use of proteins, such as *B. thuringiensis* toxins, that are encoded by a single gene. Moreover, the molecule that is expressed has to remain active after ingestion of the plant tissues by the insect. Continuing research on new sources of resistance is essential for the long term control of insect pests. In a first step, studies on the expression and potential of the new insecticidal genes can be performed in model plants such as tobacco and *Arabidopsis thaliana*, as recently proposed by Santos et al. [72]. In a second step, the selected gene(s) must be introduced into the target crop. Very often, transformation procedures work with cultivars which are not elite commercial varieties and therefore, when a transformation event has been shown to improve the efficacy of insect control, a backcrossing process must take place. In another step, field trials must be performed in different locations and for several years to demonstrate that the characteristics of the new lines are identical to that of the original elite cultivar. These different steps explain why the development of insect-resistant crops is a long term process, and why these programs are mainly devel-

oped in private companies in industrialized countries. Part of the contribution of transgenic crops to a safer and more environmentally friendly crop protection system will be taking place in developing countries where crops (cotton and rice for example) requiring high insecticidal treatments are grown. However, the introduction of insect-resistant crops must be accompanied by a resistance management strategy and, even without taking regulatory issues and intellectual property rights (which are not the aims of this review) in consideration, the delivery of this new technology to these countries will be more challenging. Another factor affecting the future of these crops is the public acceptance of products derived from transgenic plants. Better consumer information is necessary to allow a well-informed decision based on the comparison of the potential benefits of transgenic plants with the reliance on chemical insecticides.

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