
AN OVERVIEW ON TRANSGENIC PLANTS FOR INSECT RESISTANCE

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ABSTRACT

*Insect-resistant plants may be created via plant genetic engineering, which involves introducing and producing entomopathogenic proteins in the plant. There have been two major approaches to investigating such plants. Plant genetic engineering allows for the development of insect-resistant plants via the insertion as well as expression of entomopathogenic proteins in planta. There are two major methods for obtaining such plants that have been investigated. The first includes the usage of delta-endotoxin coding sequences derived from *Bacillus thuringiensis* bacteria. Plant-derived genes, including those producing enzyme inhibitors and lectins, are used in the second method. Much effort is being put in across the globe to find plants of various kinds that express such genes and are resistant to insect pests. This overview discusses ongoing research initiatives as well as an evaluation of the status and issues faced on the path to commercialization of transgenic plants.*

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. 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[1].

*1.2. Use of *Bacillus thuringiensis* d-endotoxins:*

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 (Germany). *B. thuringiensis* is a gram positive bacterium that synthesizes insecticidal crystalline inclusions during sporulation. The crystalline structure of the inclusion is made up of protoxin subunits, called d-endotoxins. Most *B. thuringiensis* strains produce several crystalline proteins (Cry proteins), each of which shows a rather narrow host range . At least 90 genes encoding protoxins from a wide range of *B. thuringiensis* isolates have been isolated and sequenced. The genes were first classified in different classes cryI, cryII, cryIII 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. The d-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. *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. However, due to their low field persistence, the use of *B. thuringiensis* sprays is still relatively limited[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. 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[3].

1.2. 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 synthesized 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. 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. These molecules interfere with the growth and development of the larvae and in some cases cause the death of the insect.

Proteinase inhibitors According to their specificity, proteinase inhibitors (PIs) can be divided in four classes, inhibiting serine, cysteine, metalloid 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. In some cases, they can be induced by wounding and insect attack. Diet incorporation assays or *in vitro* inhibition of digestive proteases studies have demonstrated the potential of PIs to interfere with insect larval growth. In addition, the expression of an antisense ProSystem in gene in transgenic tomato decreased the ability of the plants to produce PIs and consequently reduced resistance towards *Manduca seta* larvae[4]–[6].

1.2.1. a-Amylase inhibitors :

The common bean, *Phaseolus vulgaris*, contains a family of related seed proteins (PHA-E and -L, arcelin, and a-amylase (a-AI)). PHA-E and -L are classical lectins with strong agglutinin activity, but a-AI forms a complex with certain amylases and is supposed to play a role in plant defence against insects. The introduction and expression of the bean a-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*. In addition to these pests of stored grain, 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 a-AI gene to Azuki bean conferred resistance to three species of bruchids.

1.2.2. 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, 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. 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[7].

1.2.3. 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 fecundity of the aphid *A. solani*. However, the expression of a chitinase of insect origin in transgenic plants seemed to be more effective in causing larval mortality to a beetle, *Oryzaephilus Mercator*.

1.2.4. 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 and against thrips (*Frankliniella* spp.) predation in alfalfa. A recombinant *Manduca sexta* insect chitinase purified from transgenic tobacco plants was toxic for the merchant grain beetle *Oryzaephilus Mercator* when administered orally in the diet at a level of 2%. These data indicate that insect chitinases are a potential factor of resistance and might be more potent than chitinases from other sources[8].

1.2.5. 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, particularly 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 cutworm (*Agrotis ipsilon*), a corn pest. *Streptomyces* cultures secrete a cholesterol oxydase active against the boll weevil (*Anthonomus grandis*). These proteins are highly toxic (within the same range as *B. thuringiensis* toxins) and could be new interesting sources for engineering resistance[9].

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. Recently, with *B. thuringiensis* based insecticides, resistance has developed in field populations of *Plutella xylostella* and different insects have developed resistance to *B. thuringiensis* toxins in laboratory conditions.

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.

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*. In a second step, the selected gene(s) must be introduced into the target crop. Even if legal problems and intellectual property are ignored, the introduction of insect-resistant crops must be accompanied by a resistance management plan, and bringing this new technology to these nations will be more challenging (which are not the goals of this review). The social acceptability of transgenic plant-derived products is another factor affecting the future of these crops. Better consumer information is needed in order for consumers to make an informed decision based on the potential benefits of transgenic plants over the usage of chemical pesticides.

The toxin's constitutive expression in transgenic plants may put a lot of pressure on insect populations. Particular promoters may be employed to restrict insect exposure to the toxin, which could be produced in specific sections of the plant that the insect is attacking (tissue-specific), during the most vulnerable development stages (temporal-specific), or in response to insect eating (wound-specific). This tailored production of the insecticidal gene may potentially help transgenic plants gain societal acceptability. To suppress the Colorado potato beetle, for example, toxin does not need to be produced in potato tubers but just in leaves. Understanding gene regulation will be very helpful in creating tailored expression. However, transgenic crop deployment is still relatively new, and recommendations are mostly based on theoretical models. It goes without saying that there will be varied needs for each crop, insect, and location. The high dose/refuge approach requires a concerted effort on the part of biotechnology firms and farmers. It's important to remember that the integration of transgenic crops into conventional agricultural systems is still in its early stages. The survival of these plants is contingent on their correct usage and the establishment of appropriate standards.

This explains why proteins produced by a single gene, such as *B. thuringiensis* toxins, are used. Furthermore, the expressed molecule must stay active after the insect has consumed the plant tissues. The long-term management of insect pests necessitates ongoing study into novel sources of resistance. In the first stage, researchers may look at the expression and potential of novel insecticidal genes in model plants like tobacco and *Arabidopsis thaliana*. The chosen gene(s) must then be injected into the target crop in a second phase. Even if legal and intellectual property issues are overlooked, insect-resistant plants must be supported with a resistance effective management, so introducing this new technology to these countries will be more difficult. Another element influencing the future of these crops is the societal acceptance of transgenic plant-derived goods. In order for consumers to make an educated choice based on the possible advantages of transgenic plants versus the use of chemical pesticides, better consumer information is required[10].

2. DISCUSSION

Plant genetic engineering enables the creation of insect-resistant plants by inserting and expressing entomopathogenic proteins in the plant. There have been two main techniques for getting such plants explored. The use of delta-endotoxin coding sequences obtained from *Bacillus thuringiensis*

bacterium is the first. In the second approach, plant-derived genes, such as those that produce enzyme inhibitors or lectins, are utilized. In impoverished countries where crops that need extensive insecticidal treatments (cotton and rice, for example) are grown, transgenic crops will contribute to a safer and more environmentally friendly crop protection system. However, the introduction of insect-resistant crops must be preceded by a resistance management plan, and even if legal concerns and intellectual property rights are ignored, bringing this new technology to these nations would be more challenging (which are not the goals of this review). The social acceptability of transgenic plant-derived products is another factor affecting the future of these crops. Better consumer information is needed in order for consumers to make an informed decision based on the potential benefits of transgenic plants over the usage of chemical pesticides. Field experiments must also be conducted in various places and over a period of time to show that the new lines' features are similar to those of the original elite cultivar. These many stages explain why insect-resistant crop development is a lengthy process and why these projects are mostly conducted in private businesses in wealthy nations.

3. CONCLUSION

Plants employ a variety of methods to defend themselves against insects in nature. Insects are poisoned by a variety of secondary metabolites, but their biosynthesis routes are seldom studied or are too complicated to be utilized to develop insect-resistant plants. Transformation methods often operate with cultivars that are not elite commercial varieties, necessitating a backcrossing process when a transformation event has been proven to enhance pest control effectiveness. Field experiments must also be conducted in various places and over a period of time to show that the new lines' features are similar to those of the original elite cultivar. These many stages explain why insect-resistant crop development is a lengthy process and why these projects are mostly conducted in private businesses in wealthy nations. Transgenic crops will provide a contribution to a safer and more ecologically friendly crop protection system in poor nations where crops that need heavy insecticidal treatments (cotton and rice, for example) are produced. However, the implementation of insect-resistant plants must always be accompanied by a resistance management strategy, and delivering this new technology to all these countries will be more difficult even if regulatory issues and intellectual property rights are not taken into account (which are not the goals of this review). Another element influencing the future of these crops is societal acceptance of transgenic plant-derived goods. Better consumer information is required to make an educated choice based on the possible advantages of transgenic plants against the use of chemical pesticides.

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