

Genetic Improvement Potential and Constraints of Crops Towards Insect Resistance

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ABSTRACT: *Transgenic resistance to insects has been shown in plants expressing insecticidal genes such as δ -endotoxins from *Bacillus thuringiensis* (Bt), protease inhibitors, enzymes, secondary plant metabolites, and plant lectins. While transgenic plants with imported Bt genes have been implemented in many crops on a worldwide basis, the alternative genes have received much less attention. The protease inhibitor and lectin genes primarily influence insect growth and development and, in most cases, do not result in insect death. The effective concentrations of these proteins are considerably higher than the Bt toxin proteins. Therefore, the potential of some of the alternative genes can only be realized by deploying them in conjunction with traditional host plant resistance and Bt genes. Genes giving resistance to insects may also be distributed as multilines or synthetic varieties. Initial indications from deployment of transgenics with insect resistance in various cropping systems in USA, Canada, Argentina, China, India, Australia, and South Africa indicate that single transgene products in conventional cultivar back-grounds are not a formula for sustainable pest control. Instead, a far more complicated strategy may be required, one which may entail deployment of a mixture of various transgenes in diverse backgrounds. Under varied climatic circumstances and agricultural systems of tropics, the success in the use of transgenics for pest control may require decentralized national breeding pro-grams and numerous small-scale seed businesses. While many trans- genic crops containing insecticidal genes have been introduced in the temperate areas, relatively little has been done to utilize this technology for enhancing agricultural yield in the harsh conditions of the tropics, where the need for increasing food supply is most urgent. There is a need to establish suitable methods for deployment of transgenics for pest control, bearing in mind the pest spectrum involved, and the impacts on nontarget species in the environment.*

KEYWORDS: *Antibodies, CaMV35S, DNA, Genetic Transformation, Insect Pest.*

1. INTRODUCTION

There is an ongoing need to boost food production, especially in the Asian, African and Latin American emerging nations. Insect-pest losses are one of the single biggest crop productivity limitations estimated at 14% of overall agricultural output. Furthermore, insects serve as vectors for several plant diseases. At now, the yearly worldwide expense of trying to minimize pesticide harm is about 10 billion US dollars. In addition to detrimental impacts on non-target species and the environment in general, large pesticide use for insect control leads in hazardous residues in food and food items. In addition, in marginal cultivation systems, the cost-benefit ratio of these methods may easily be adverse, especially when other variables, such illnesses or drought, are also limited in crops output[1].

Insect pest losses may be successfully reduced by resisting insects in the host plant compared with other main limitations of crop production such as poor soil fertility and drought. The capacity to extract and modify individual genes by using recombinant DNA technology and the ability to implant certain genes in a selected variety have opened a new age of targeted plant breeding. In the course of the last two decades' considerable progress has been achieved in introducing foreign genes into plants, providing possibilities to alter crops in order to enhance yields, increase resilience and improve nutritional quality against biotic and abiotic stress. Genes that encoded *Bacillus thuringiensis* (Bt) δ -endotoxins were cloned in the early

1980s and in the mid-1990s, genetically engineered plants with the resulting insect resistance were created. In this article we are focusing on candidate genes that confer resistance on insect pests and review current progress in the development of transgenic insect resistance products and their limitations to evaluate the future potential and genetic improvement of crops in developing countries to enhance the livelihoods of the poor.

1.1 Crop Plants Genetic Transformation:

Tissue culture and transformation protocols are one of the most essential components for effective transgenic crop production. The main components for transgenic plant development are:

- Preparation of gene structures and transformation by appropriate vectors,
- Effectiveness of transformation techniques for introduction of genes in crop plants,
- Rehabilitation and propagation of transgenic plants,
- Molecular and genetic characteristics of transgenic plants for stable and effective gene expression
- While many techniques to the iterative transformation have been attempted successfully, only four methods are widely utilized and allow scientists to introduce genes to a broad variety of agricultural plants.
- The transfer of agrobacterium-mediated gene,
- The bombardment of DNA or biolistic with micro-projectiles,
- The microinjecting of DNA, and
- The direct transmission of DNA into isolated protoplasts. The first two methods were utilized very effectively of these strategies.

Tumefaciens agrobacterium was extensively employed to convert the desired genes into crops. It is a bacterium with a soil population that is involved in the development of gall at wound sites in several dicotyledonous plants. The presence of a large Ti (tumor-inducing) plasmid in virulent agrobacterium strains is responsible for this tumor-induction capacity. Also, root-causing megaplasmids are detected in virulent strains of "hairy root" disease agent *Agrobacterium Rhizogenes*. The plasmids Ti and Ri and the molecular biology of the induction of the gall and hairy root have been carefully investigated. Agro-bacterial-mediated transformations occur when an independently replicated Ti plasmid is incorporated into an *A. tumefaciens* cell, which then infects a cell of the plant and transfers the T-DNA that contains a gene of interest into the chromosomes of the host plant's cells[2].

Genetically modified DNA may also be directly inserted into nuclei of single embryogenic cells that can regenerate plants in cell culture. This involves micromanipulation of individual cells or tiny colonies of cells under the microscope and accurate injection using a thin glass micro-pipette of small quantities of DNA solution. Cells or clumps injected into cells are later grown and regenerated into plants in *in vitro* cultivation methods.

The tungsten or gold particle micro-projects are coated with the insertion of DNA using the tungsten bombardment technique and blasted into cells/tissues capable of later regeneration of the plants. Acceleration of hefty DNA-coated micro-projects delivers genes into nearly every cell and tissue type. DNA-coated particles enter the plant cells, a tiny percentage of the cells are included in the DNA and the transformed cells are chosen for plant regeneration[3].

The cell wall of target cells is destroyed via enzyme treatment and the cells are confined by a plasma membrane during protoplast transformation. The DNA may be added to the cell suspension, which can be introduced via the influence of the plasma membrane by polyethylene glycol or through the proto-plastic suspension of an electrical current. The DNA

is integrated into a few cells' genome. To select the trans-formed protoplasts and cell colonies that grow from them, an appropriate marker may be introduced. The operon Cry2Aa2 in tobacco chloroplasts resulted in a Bt protein concentration in mature leaves of up to 45,3% of the total protein resulting in 100% death of cot-ton bollworm and beet army-worm[4].

1.2 Genesis Expressions Genesis:

Efficient genetic engineering depends on being able to produce a gene product in the appropriate tissues at the correct level of expression at the right time. This may be done by constructing gene constructs that contain promoters and/or regulatory elements for transcription that regulate gene expression levels, locations and times. The absence of promoters that can provide a high level of gene expression at that level of specificity in the crop-species of interest was a key limitation in developing successful transgenic goods. Traditionally transgenic expression is driven by strong component promoters like as CaMV35S and Actin 1. While CaMV35S was extensively utilized in a number of dicotyledonous systems, it is low in monocotyledonous systems. In addition, it is difficult to anticipate the pattern of CaMV35S promoter activity in various tissues of transgenic plants. In general, monocot promoters have been shown to be more active in monocot tissue than in dicot tissue[5].

Recently, tissue-specific promoters have been used to drive transgenic expression only in pith tis suit. Carboxylase phosphoenolpyruvate (PEPC) may be utilised in green tissue for gene expression. Insect-resistant transgenes should only be expressed from the crop-yield-potential viewpoint in those organs likely to be attacked by insects. If not, plants may be extremely resistant, but metabolic costs may significantly decrease crop production. It also lowers the likelihood of unanticipated detrimental impacts on non-target species. Often, findings on gene expression levels may not be extrapolated from one species to another and each crop should be evaluated by a set of promoters. While component promoters like CaMV35S are efficient in ensuring high levels of gene expression, these expressions are not only unnecessary, but may have unforeseen detrimental effects for non-target species in certain instances. On the contrary, a more focused expression of insecticidal genes may constitute an essential component to the development of insect resistant transgenic plants employing a tissue and organ-specific promoter.

Transposon-mediated transgene repositioning is an interesting approach for generating plants without selectable markers and T-DNA inserts. A significant number of transgenous insertions in the genome may be made by utilizing a minimum number of transformation events to take advantage of positions in the genome that can contribute to greater levels of expression. The maize ubiquitin promoter Cry1B gene produced between the minimum terminal inverted maize AcDs transposon system repetitions were cloned in the 5r untranslated region of the gfp gene utilized as an excision marker. The findings have shown that transposon-mediated gene relocation is a potent technique for producing T-DNA integration without site-free transgenic plants and taking use of advantageous positions on the plant genome[6].

1.3 Enzymes:

Several transgenic enzymes shown resistance to lepidopteran insects. Streptomyces cholesterol oxidase is highly toxic for cotton boll weevil while polyphenols oxide and peroxide increase the inhibitory effects of 5CQA and cholorogenic acid by oxidizing the dihydroxy groups with ubiquinones that covalently tie proteins, peptides and amino acids into the nucleophilic groups. Mechanical wounding and injury to insects resulted in a temporary increase in polyphenol oxidase activity. However, after wounding, insect damage or

administration of methyl jasmonate, there is no systemic induction of the enzyme. Soybean lipoxigenase has also shown harmful effects on insects and was proven in transgenic plants, however insect resistance has not been established. The use of the bacterial isopentyl transferase gene involved in *Nicotiana plumbaginifolia* cytokinin production lowers *M. sexta* larval feeding by 70 per cent and delays the growth of peach potato aphid, *M. persicae*. Zeatin and zeatin-riboside levels are about 70 times higher in leaves that survive in PI-II-ipt plants after hornworm feeding. Exogenous use of zeatin in the PI-II-ipt leaves increases resistance to cigarette and totally prevents the normal growth of the green fish aphid. Transgenic chitinase tobacco plants also showed resistance to many insects.

1.4 Antibodies:

Antibody-based genes may also be used for genetic transformation of agricultural plants. The function of important insect proteins as control agents against nematodes, pathogens and viruses may be blocked by single chain antibodies. This method to insect management has the benefit of enabling a degree of selection for particular effects so that insect pests are targeted, not beneficial species. The discovery of a mechanism for transport from transgenic plants to the insect hemolymph removes a major limitation on the transgenic approach to crop protection[7].

1.5 Transgenic Crops Limitations And Risks:

Recent biotechnological advances in plants provide both possibilities and difficulties. Insect migration from sprayed fields to transgenic crops may affect the close proximity between transgenic plants and sprayed non-transgenic agricultural fields and the resulting increases in the pest burden can decrease transgenic advantages. Bt poisons have been extensively employed for many years as "natural" insecticides without reports of spontaneous resistance development in the population of insects. However, when the prevalence of Bt toxins in the environment dramatically increases, pressure on insect populations to develop resistant biotypes may rise substantially. Evidence of this remains un-conclusive and rigorous monitoring is needed before the large-scale use of transgenic crops in subsistence farming. One way to address these issues is to create a new generation of transgenic with superior genes and utilize gene combinations to postpone resistance development in insect populations. Problems that limit the usefulness of transgenic crops in insect monitoring include:

- limited performance;
- secondary pest problems;
- sensitivities of insects;
- resistance to and development of new biotypes;
- approximate gene expression influences;
- environmentally friendly gene escape;
- impacts on non-target organisms;
- biosafety of foods from transgenic crops.

1.6 Performance Restrictions:

The effects of Bt toxins on insect death cannot be the same as those of synthesized pesticides and thus farmers must be informed on the effectiveness and mechanism of action of transgenic crops. Transgenic crops cannot tolerate high insect densities in certain seasons and thus rigorous insect populations monitoring should be an important component in the management of transgenic crops by insect pests. National governments may need to create laws requiring trading firms to guarantee that this kind of monitoring is routinely carried out

across all crops. The benefit of transgenic plants can best be appreciated if they are used as an insect pest control component. In certain instances, the yield of *H. armigera* resistant transgenic cottons is lower than that of conventional types. The actual advantages must thus be viewed in connection to the decrease in frequency and dose of the application of pesticide. Bt maize has usually shown a lower protection value than the existing seed premiums in Indiana, USA. Therefore, stricter science on the performance of transgenic plants with resistance to insects in a really integrated insect pest control system is urgently needed to quantify its long-term performance and accurately predict its interaction with different environmental circumstances. The present gene promoters control the gene expression in green tissue and the expression in young plants is more pronounced. Some insects, such as bollworms, pod borers and stem borers, penetrate a plant tissue that is not fully chlorophyllated and may lack the toxin proteins. Insufficient toxin expression may lead to resistance development and thus it is necessary to ensure that the toxins are produced in sufficient quantities at the site of insect injury and feeding[8].

1.7 Secondary Insect Pest Issues:

In the absence of pesticide treatments for the management of pests, large-scale cultivation of insect-resistant transgenic plants may result on secondary nontarget insects becoming significant constraints in crop output. Consequently, spraying may have to be resumed to manage the secondary insect pests. Chemical sprays used to manage secondary pests may destroy natural adversaries, compensating transgenics for one of their benefits. Most field crops are attacked by multiple insect species, and the secondary insects may take on a significant pest status in the absence of a request from the main insect pests. Bt toxins also may not work for some insect pests such as leaf hoppers, mirid bugs, root feeders, mites, etc. This may offset some of the anticipated benefits of insect-resistant transgenic plants. The management of stinkbugs is required in bollworm-resistant transgenic cotton. Gens that can be used to control insects that are not sensitive to Bt toxins need to be identified. Genes with a wide range of activities will be advantageous if such genes do not affect the activity and abundance of beneficial and non-target species[9].

1.8 Development of New Biotypes:

Insect-resistant cultivars produced from traditional breeding have demonstrated no direct connection with the development of novel biotype insect resistance, for example the deployment of fly-resistant cultivars has not resulted in new insect biotypes being developed in wheat. With Greenbug, however, breeding efforts always struggle to keep pace with the development of novel biotypes. Only 3 of the 11 greenbug biotypes showed a connection in sorghum between resistant hybrids and the emergence of new biotypes. The interactions between insect plants are very particular and future attempts should concentrate on the use of the most efficient resistance genes or utilise several genes to slow down the development of new biological kinds of insects. Insects' capacity to overcome host plant resistance is always a significant danger. The insects are exposed to toxin proteins in transgenic crops throughout the feeding cycle / season and thus the populations of insects are under constant selection pressure. The majority of the previous transgenic plants are controlled by Bt genes such as CaMV 35S and this approach may lead to resistance development in the target and in the non-target insect, since the toxins are expressed in all sections of the plant. The generation of toxins may also decrease throughout the growth season. Low dosages of poisons remove the most susceptible people in a group and allow a population to build resistance much more rapidly. Since most Bt toxins are similarly active, resistance to one toxin may potentially lead to cross-resistance to other toxins. There are indications, however, that insects chosen for one Bt toxin tolerance may not be resistant against other Bt toxins[10].

2. DISCUSSION

Genetic engineering may potentially have unanticipated impacts on a plant's environment. Consumers are grasping this basic but unexpected phenomena and emphasize a great deal of the worry about genetically modified crops that is increasingly becoming more general concerns about any food product based on biotechnology. New food innovations obviously need to be thoroughly evaluated for their possible allergens, toxins and antimetabolisms in a manner comparable to contemporary pharmaceutical products. Most Bt toxins are insect-specific since they are triggered in the alkaline intestine media. In the gastrointestinal system of animals, including humans, there are no particular Bt protein receptors. The Bt proteins are quickly destroyed in vertebrates by stomach fluids. The composition of Bt tomatoes and potatoes is not significantly changed. Therefore, transgenic Bt tomatoes in comparison with normal tomatoes are deemed to represent no extra danger to human and animal health. A number of issues relating to the safety evaluation of transgenic food would, however, need additional consideration. The seed of the Bt-transformed cotton lines is equally nutritious as the seed from parent lines and other commercial cotton types in terms of composition. In transgenic cotton seed, the processing eliminates more than 97 percent of Bt proteins. CryIA(b) protein dissipated readily or grown into the soil on the surface of the soil and was not found in transgenic silage. Intact maize silage transgenes are unlikely to survive in sheep rumen substantially. DNA released from the food in the mouth may, however, maintain sufficient biological activity to convert competent oral bacteria. Histopathic- logical effects were seen in mice and rabbits in the intestinal mucosa, but systemic adverse effects were not seen after oral treatment. There are no survival and body weight differences in broilers treated with Bt transgenic and non-transgenic maize in the meshed or pellet- ted diets.

Several groups of proteins that contribute to systems of natural defense in plant crops are allergens or suspected allergens and may be harmful to human animals. These include alpha-amylase and trypsin inhibitors, lectins and proteins linked to disease. Trade is thus established between natural pesticides produced by transgenic plants, varieties of conventional breeding programmes, synthetic insecticides, mycotoxins, etc. Rats fed on a semi-synthetic diet with pure trypsin cowpea inhibitor have demonstrated a modest decrease in weight growth despite the same food consumption. Most CpTi were quickly break down in the digestive system, and their presence in the diet resulted to a small rise in faecal nitrogen but not urine. After transferring the CpTi gene into food plants, the nutritional cost for enhanced insect resistance is quite modest in the short run. The amount of GNA lectin expression that protects plants does not decrease the development of rats; it has a poor impact on the weight and length of the small gut and a little hypertrophy of the tissue. The brush border enzyme activity has been impacted, sucrase isomaltase almost halved and there was a substantial rise in alkaline phosphatase and aminopeptidase activity. Wheat germ (WGA), apple and nettle agglutinins interfere with metabolism to different degrees. A novel gene may also be introduced in a crop to bring new allergens that are not usually present in non-transformed plants. Biotechnology can introduce new proteins in plant, bacterium and virus food crops with unknown allergic properties. If the proteins added come from recognized sources, the allergenicity of genetically engineered plants may be predicted and evaluated easily. Eight common allergens and 160 fewer allergens were identified, and the transfer of genes associated with those allergens could certainly be avoided by scientists. While no documented serious effects of transgenics on mammals have been reported to date, extensive studies on a case by case basis should be undertaken before a transgenic crop is released by farmers for large-scale cultivation. On the other hand, genetic transformation can reduce the allergy of conventional foods. Anti-sense technology, for example, can hold promises to reduce the dramatic allergy of peanuts and other nuts.

3. CONCLUSION

Many agricultural practices for risk management are in place. The risk of the transfer of genes from a transgenic crop to a weed, e.g., from canola to weedy mouth, can be managed when a herbicide is sprayed with another mode of action. Crop rotations can also be used to control weeds of this kind. By producing seeds under rigorous certification processes to detect crop weed hybrids in plant production plots, the danger of introduction of a viable hybrid among transgenic plants and weedy relatives may be dealt with. Gene transfers may be prevented between the same species by maintaining a safe distance between the neighbouring plots. Such information is accessible for most farmed crops to prevent outcrossing. Serious scientific investigations should be performed before the introduction of a transgenic plant with particular genes in places where gene transfer is more likely, e.g. in a plant's centre of origin. Varieties or crops that are likely to be transported to the following crop season, or to infect the same crop next year, may be replaced with reduced or no seed transportation to the next season. The effectiveness of many of these activities depends, however, on collective community effort and/or strong national law.

The ideal transgenic technology should be economically feasible, ecologically friendly, simple to deploy in many agro-ecosystems and have a broad range of activities to combat the target insect pest. The sites of insects which have developed resistance with conventional insecticides should also be unhealthy for natural enemies and untargetable organisms, must be flexible enough to allow ready deployment of alternatives and should preferably produce acute rather than chronic effects on target insects. Some of the requirements can be accomplished by using antibody-based genes. The function of critical insect proteins may be blocked by single-chain antibodies. Population-expressed antibodies or antibody fragments have been shown to be promising insect control agents against nematodes, diseases and viruses. This method of insect management would benefit by reducing a certain degree of selection for particular effects such that insect pests, but not the beneficial species, are targeted. The discovery of a delivery route for poisons to insect hemolymph from transgenic plants eliminates a fundamental limit in the transgenic approach to crop protection.

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