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POPULAR SCIENCE ARTICLE

APPLICATION OF MOLECULAR TECHNIQUES FOR PEST MANAGEMENT AND IT'S LIMITATIONS

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The world population will increase to 7.5 billion by 2020 ^[1]. One of the important problems in agriculture is the loss of agricultural produce due to attack by insects and pests. Losses in agriculture due to a diversity of potentially pathogenic micro-organisms, phytophagous insects and other invertebrate pests signify one of the major constraints to crop productivity in tropical and temperate regions of the world. The present day pest control is mainly dependent upon the use of insecticides. A fundamental contributor to the Green Revolution has been the development and application of pesticides for the control of a wide variety of pests that would otherwise reduce the quantity and quality of food produce. The use of pesticides amplified since the 1960's ^[1]. Due to indiscriminate use of insecticides, it has developed high levels of resistance to conventional insecticides. As excessive use of pesticides result in harmful effects on beneficial organisms, they also leave residues and result in environmental pollution. Therefore, it is necessary to develop more environmental friendly agricultural techniques that will not produce harmful output such as pesticide residues.

To overcome the limitations of conventional breeding the recombinant DNA technology may be developed. Genetically engineered crop plants enhanced insect resistance have revolutionized agriculture and lead to a reduction in pesticide usage and lower production costs worldwide. Advances in genetic transformation and gene expression

during the last decade boost up crop improvement using genetic engineering, of which protection of crops against the insects is a major goal. The possibility of this technology has now been widely recognized therefore, it is important to characterize insecticidal proteins and their genes from our indigenous crops in order to strengthen and broaden our gene bank for pest control operations. The first success came in 1987 with the endotoxin gene from *Bacillus thuringiensis* (Bt) being transferred to tobacco plants. Since then, *B.thuringiensis* genes have been transferred to a number of other crop species such as cotton, rice, maize, canola etc. The availability of different insecticidal proteins and their genes from different plant species will make it easier to use one or more genes in combination to develop resistant crop plants against biotic and abiotic stresses.

Therefore it is important to isolate and identify such genes from indigenous crop plants and transfer them for greater expression in other crops. Development of transgenic plants, engineered for insect resistance has added a new dimension to crop improvement.

Biotechnology in Integrated Pest Management: Integrated Pest Management is today a widely accepted tactic to reduce overdependence on chemical insecticides and their potentially negative impact on environment and socio-economic conditions. Biotechnology has considerable potential to contribute towards sustainable biological elements of IPM. Biotechnology development to date has been focussed at more conventional models for pest

control technologies. It has enormous potential to improve pest management. Biotechnological research has been now focussed on improving natural enemies of pests as pest control agents. Natural enemies includes bacteria, viruses, fungi, nematodes, predators, etc. We already know about *Bacillus thuringiensis* (Bt), which is widely used as a biopesticidal formulation to control caterpillar and beetle pests of crops, and flies which are disease vectors^[1].

The second principle area of biotechnology for pest control has been the development of crop varieties resistant to pests and diseases. This has concentrated on incorporating insect and virus resistance into the plant genome. In addition, modification of the genome of plant associated microorganisms has been monitored as a strategy to confer insect resistance to plants.

Transgenic Plants: Transgenic plants are plants which possess one or more additional genes. This is attained by cloning additional genes into the plant genome by genetic engineering techniques^[1]. The added genes expose resistance to pests.

Applications of Molecular Techniques in agriculture: Many economically important genes were engineered into crop plants worldwide. But a major influence has been made in the field of crop protection. Here, plants transformed for pest resistance in some detail.

Insect-resistant Transgenic Plants Expressing Bt Toxin: *Bacillus thuringiensis*, a natural soil bacteria that secretes a lethal endotoxin. Bt toxins are highly effective for many pest organisms, like Lepidopterans, Coleopterans, Dipterans and other interrelated species, but not toxic to mammals and most other non-target organisms. The use of genes encoding endotoxins from *Bacillus thuringiensis* is now a well-established technology for producing transgenic plants with improved resistance to the larvae of lepidopteran insect pests. Regarding mechanism of bacterial toxin action, when the insect larvae feed on transgenic plant, crystals and spores are ingested into the midgut of the insect. Since the pH is alkaline in nature, so the crystals develop toxic to insect midgut leading to septicaemia. Bt toxins have been commercialized including maize, tomato and potato. The adoption of Bt crop varieties by farmers has been rapid returning the benefits of these crops such as decreased insecticide use, lower production costs and higher yields.

B. thuringiensis, a Gram-positive soil bacterium, produces a proteinaceous parasporal crystalline inclusion during sporulation. There are two chief categories of Bt toxins: Cry and Cyt. The Cry toxins are divided into three larger families that are not related phylogenetically. The leading Cry family is the three domain family, and genes from this family are present in the majority of commercialised Bt crops. The larvae of insect orders primarily affected by BT toxins are Lepidoptera (butterflies and moths), Diptera (mosquitoes) and Coleoptera^[2]. However, BT toxins are not toxic to people, wildlife, or most beneficial insects and therefore the opportunities for biological control are enormous. In 2011, planting of BT cotton in India exceeded the historical milestone of 10 million hectare for the first time and occupied 88% of the recorded 12.1 million hectare cotton crops^[3].

Management of Resistance to Bt Crops: There are two main tactics for management of insect resistance to Bt crops: refuge and pyramiding.

Refuge: The main tactic for delaying evolution of resistance to Bt crops is the refuge strategy. Farmers are mandated to maintain an abundance of host non-Bt crops as a refuge surrounding their Bt crops. The theory behind this strategy is that any Bt resistant larvae that arise on the Bt crops will mate with susceptible individuals from neighbouring non-Bt crops^[1].

Pyramiding: Major strategy to combat the development of Bt resistance is gene pyramiding. The development of second generation Bt cotton that has at least two Bt toxins such as the Monsanto Bollgard II cotton variety^[4]. The success of uniting multiple Bt genes for resistance management is depending on the individual toxins having different targets to prevent cross resistance.

Insect Pest and Biotypes Identification: DNA markers strongly linked to the gene of interest can be used at any crop stage for testing the presence of the gene rather waiting to observe its phenotypic manifestations^[5]. Also, identification of mealybug pest species in Egypt and France has been studied using a DNA barcoding approach^[6]. Number of biotypes also identified with help of biotechnology.

Biotechnology in Biopesticide Development: Today there are above a hundred commercial biological control products on the market, and many more are locally produced and supplied for specific productions systems. However, most commercial biological control have focused on

insect pathogens, because of their relative ease of mass production and their capacity to be used in the same manner as formulated chemical insecticides. Bt has been the principle target of product development, and accounts for most sales in the 75 million global market for biological control products^[1]. However, this is only less than one per cent of global pesticide sales. As a product, Bt is valuable in IPM systems because it is much less harmful to predators and parasites than broad spectrum chemical insecticides. Therefore, it can be substituted for chemical products in "insecticide treadmill" situations and will concede the recovery of natural enemy populations. A key advantage of biological agents relative to chemical pesticides is their ability to both kill pests and reproduce at the expense of pest (numerical response) thereby giving some control in the future pest generations. Bt focused more on maximizing the effect of its insect killing toxin. In other words, its commercial development has focused on using it like a chemical insecticide and not as a living biological control agent.

Fungal Resistance: Fungal pathogens cause several important diseases in crop plants. For many years, application of fungicides is the only efficient strategy for their management. Conventional breeding for fungal disease tolerance has not proven to be effective.

Plant genetic manipulation through expression of either unique proteins from foreign organisms or overexpression of a part of their own defensive resource for disease resistance has become a reality in the past decade. Expression of chitinases and glucanases, like other pathogenesis-related-proteins, are induced in host plants in response to pathogen attack to degrade the invading fungal cell wall. Therefore, different classes of chitinases and glucanases of diverse source have been engineered into a number of transgenic plants with varied degree of success against different fungal pathogens.

Under non-induced circumstances the transgene was silent but upon infection by virulent fungal pathogen, cryptogin production was stimulated which coincided with the fast induction of several defense genes at and around the infection sites. Induced elicitor production resulted in a restricted necrosis that restricted further growth of the pathogen. The transgenic plants displayed enhanced resistance to fungal pathogens that were unrelated to *Phytophthora*

species, such as *Thielaviopsis basicola*, *Erysiphe cichoracearum* and *Botrytis cinerea*^[7].

Bacterial Resistance: Recent development in the understanding of plant-pathogen interactions enables the use of genetic engineering for the rational creation of bacterial disease-resistant plants. To date, the strategies engaged for developing transgenic plants resistant to bacteria are: production of antibacterial proteins of non-plant origin, inhibition of bacterial pathogenicity or virulence factors, enhancement of natural plant defenses and artificially encouraged programmed cell death at the site of infection. Antibacterial proteins of non-plant origin include lytic peptides, lysozymes and iron sequestering glycoproteins. Lytic peptides are small proteins with an amphipathic helical structure whose effect is to form pores in bacterial membranes. Cecropins have been expressed in transgenic potato and tobacco and attacins in apple plants^[1]. Lysozymes are ubiquitous enzymes with precise hydrolytic activities directed against the bacterial cell-wall peptidoglycan. Three different lysozyme genes obtained from different sources have so far been expressed in plants. Inhibition of bacterial toxins by cloning and expression of a gene whose product either inactivates or is insensitive to bacterial toxin has been successfully employed to develop transgenic tobacco and bean plants^[8].

Limitations: Transgenic technology can be very attractive and has many advantages compared with other conventional procedures. But it has certain drawbacks too. Some of these are listed below.

Influence of GMOs on Environments: The gene transferred into an organism or the consequential products can actually remain in environmental leading to environmental problems. The intentional release of GMOs into the environment has led to an amplified interest in possible interactions that may occur between other organisms in the environment. Unintended genomic variations can occur as a secondary consequence of genetic modification^[1].

Gene Flow: Accidental cross breeding between GMO plants and traditional varieties through pollen transfer can contaminate the traditional local varieties with GMO genes resulting in the loss of traditional varieties of the farmers. The potential benefits of planting insect-resistant transgenic crops include reduced insecticide use and reduced crop damage. However, the innate

ability of insect populations to rapidly adapt to environmental pressures poses a severe threat to the long-term efficacy of insect-resistance. Adaptation by insects and other pests to pest protection mechanisms can have environmental and health impacts^[1].

Increased Weediness: Weediness means the affinity of the plant to spread beyond the field where it was first planted. There are apprehensions about GM crops becoming weeds. For example, a salt tolerant GM crop if escapes into marine areas could become a potent weed there. There is also fear about the development of superweeds i.e. a weed that has developed the herbicide tolerant gene due to genetic contamination with a herbicide tolerance GMO through in field cross breeding to related species or through horizontal gene transfer^[9].

Loss of Biodiversity/Reduction of Cultivars: There have been concerns about decrease in the genetic diversity in cropping systems by the development and global spread of improved crop varieties to the green revolution. This genetic erosion has happened as the farmers have replaced the use of traditional varieties with monocultures. This is expected to further intensify as more and more transgenic crops are introduced which bring in considerable economic benefits to the farmers. The relative rate of susceptibility to any unforeseen infections or destructive situations increases when single varieties are used in cropping system in place of several varieties^[1].

Changes in the Soil Ecology: Many plants leak chemical compounds into the soil through their roots. There are alarms that transgenic plants may leak different compounds than conventional plants, as and unintended sequence of their changed DNA. Opinions are that this may change the ecology of the soil in terms of functional composition and biodiversity^[1].

Conclusion: Genetically modified organisms have been developed by application of

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progressive techniques of genetic engineering. Molecular techniques use for development of pest resistant transgenic plant is Recombinant DNA technology and transformation techniques (microinjection, biolistics transformation, DNA transfer by calcium phosphate method and liposome mediated transfer). Considerable progress has been made in developing transgenic plants with toxin genes from *Bacillus thuringiensis* (Bt) in different crops. A gene for amylase inhibitor from *Phaseolus vulgaris* was engineered for high level expression in cotyledons for pest resistance. Genes for resistance against certain herbicides have been introduced into crop plants so they can survive even when exposed to herbicides. Efficiency improvement in natural enemies such as Insect parasitoids and predators is achievable with gene transfer technique. Molecular genetic identification of Insect parasitoids and predators will strengthen efficient bio-control programme. It is the need to discover new and cheap method of gene transfer technologies for developing resistant plants against pest and also improving quality of produce. GM crops offer distinctive advantages like insects, weed, disease and drought resistance, better nutritional value and higher yield. Herbicide-tolerant crops may cross-pollinate weeds, resulting in "superweeds" and challenges like this has to be taken care. However, in a significant part of the world, non-governmental organizations and the general public are hesitating or opposing the cultivation and application of GM crops. There are numerous environmental and public issues concerning the GMO's. Overall, genetic engineering of the organisms, plants and animals is accepted conditionally. The gene inserted and its products should be judiciously assessed both for human and environmental safety before release into the nature and to the public market.

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