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Current Perspectives on Transgenic Plants – A Review

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Abstract

The world's population may top nine billion people by the year 2050. To meet the needs of such a vast number of people, food production will need to be increased at the same rate or even faster. So, there is a need to use the genetic techniques to improve crops production and quality. Agricultural and horticultural crops offer substantial inputs to crop productivity, nutritional quality and human health. In recent times with advances in scientific and technological knowledge, there has been a high degree of success in crop improvement through marker assisted selection and genetic modification. Transgenic plants are the ones, whose DNA is modified using genetic engineering techniques with the main aim to introduce a new trait to the plant which does not occur naturally in the species with various benefits such as reduced environmental impact from pesticides and insecticide, increased yield, soil conservation, phytoremediation etc. However, it is now more than fifteen years since the first transgenic plants were generated experimentally. In that period there have been dramatic advances in our understanding on both basic and applied aspects of plant biology. Therefore, in the current narrative review of literature we mainly aimed describe and delineate on the current prospectives of transgenic plants, and their potential applications.

Keywords: Transgenic plants, Phytoremediation, Herbicide resistant, Virus resistant,

Introduction

Transgenic plants are the ones, whose DNA is modified using genetic engineering techniques. The aim is to introduce a new trait to the plant which does not occur naturally in the species. A transgenic plant contains a gene or genes that have been artificially inserted. The inserted gene sequence is known as the transgene, it may come from an unrelated plant or from a completely different species. The purpose of inserting a combination of genes in a plant, is to make it as useful and productive as possible. This process provides advantages like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance, against a variety of biotic and abiotic stresses. Transgenic plants can also be produced in such a way that they express foreign proteins with industrial and pharmaceutical value. Plants made up of vaccines or antibodies (Plantibodies) are especially stricing as plants are free of human diseases, thus reducing screening costs for viruses and bacterial toxins.[1]

In modern biotechnology genetic engineering is a powerful tool for transformation of individual genes. This is possible by altering the genes in an organism or between organisms by deleting or inserting a desired feature or trait. The main purpose of inserting a combination of genes in a plant is to make it more productive, disease resistant etc.[2,3]For centuries, people world over have developed new crop types by selecting plants from the available collection. This selection process formed the basis of developing new crops or plants which are now commonly known. Identification and selection of desirable traits in plants and combining these into one individual is termed as plant breeding.[4]

Currently, we know that the process of plant breeding is based on changes brought about in a plant's genetic structure. The genes in each plant cell (approx 30,000) are responsible for coding the information for a plant's phenotype. Since 1900, Mendel's laws of genetics provided the scientific basis for plant breeding. As all traits of a plant are controlled by genes located on chromosomes, conventional plant breeding can be considered as the manipulation of the combination of chromosomes.[5] Conventional plant breeding involves changing the genes of a plant so that a new and better variety is developed. The aim is to combine the favorable traits from both parent plants and exclude their undesirable traits in a singular new and better plant variety. Conventional plant breeding may also use 'wider crosses' that involve crossing species or even genera that are quite unrelated. These crosses cannot occur without help so sophisticated techniques like genetic transformation were employed.

In 1970s, with the advent of recombinant DNA Technology, it has become possible to modify the genetic information of living organisms in a new way, by transferring one or more fragments of DNA directly between organisms across species, genera or even kingdom. Organism thus developed contains a gene or genes which have been introduced artificially into its genetic makeup by using a set of several biotechnology techniques collectively known as recombinant DNA (rDNA) technology is called transgenic plant or animal as the case maybe. DNA spliced to the coding portion of the genes that serves to regulate how they function is also transferred into the host plant. The genes inserted into the host plant are called transgenes and may come from another plant of same or different species and may be from the completely unrelated kind of organism like bacteria or animals. Progress in traditional plant breeding is limited by the genetic diversity within each crop species, the diversity sometimes available from closely related species, or occasionally useful diversity created within the crop itself by inducing mutations. Often, genes for traits that could be of benefit are not found in a particular crop species, so the ability to make plants with new, desirable traits borrowed from other species represents a major technological advance over conventional breeding methods. The transgenic crop varieties made by borrowing genes from other organisms could, potentially be employed for introducing the novel traits that would be impossible to incorporate using traditional methods.[6]

The production of plants with improved quality traits such as disease resistance, prolonged shelf-life, and drought resistance by conventional breeding is extremely time consuming.[7]However, the demand for food due to an ever-expanding global population and changes ineating habits has continually increased the demand for more productive food and feed crops. In fact, the provision of sufficient food to feed an estimated 9.7 billion people by 2050,[8] and approximately 11.0 billion by 2100 is one of the major challenges of this century.[9] Genetically modified (GM) crops provide an opportunity to increase food and feed production efficiently by generating plants with higher yields and greater nutritional benefits in reasonably short times.[10]GM crops offer the possibility of expanding the accessible gene pool for breeding by overcoming sexual incompatibilities between plants and providing the opportunity to use genes with beneficial traits and regulatory elements to express genes of prokaryotic or viral origin.[11]

It is now more than fifteen years since the first transgenic plants were generated experimentally. In that period there have been dramatic advances in our understanding on both basic and applied aspects of plant biology. Therefore, in the current narrative review of literature we mainly aimed describe and delineate on the current prospectives of transgenic plants, and their potential applications.

Historical Perspectives

The foundation of plant biotechnology and the concept of transgenics can be dated back to nineteenth century when Schleiden (1838)[12] and Schwann (1839)[13] proposed the Cell Theory – “the cell as the primary unit of all living organisms” and further Haberlandt (1902)[14] predicted the cellular totipotency i.e., production of somatic embryos from vegetative cells. The concept of cellular totipotency became the key of development of transgenic crops using plant regeneration and transformation techniques. Tobacco was the first plant species to be regenerated *in-vitro*,[15] and was used to develop standardized tissue culture conditions.[16] Over the last three decades, tobacco has been established to be a plant model system for the analysis of transgene integration and stable transformation. Lederberg and Tatum (1946)[17] first discovered in bacterial system; DNA can transfer from one cell to another. This was the first report of genetic transformation in living organism. Genetic engineering is thedirect manipulation of DNA by humans outside breeding and mutations. The term “genetic engineering” was first used by Jack Williamson in his science fiction novel ‘Dragon’s Island’, published in 1951. In 1972, Paul Berg created the first recombinant DNA molecules by combining DNA of monkey virus SV40 with the DNA of lambda virus. After one year (1973), the first transgenic organism was created by Herbert Boyer

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and Stanley Cohen. Antibiotic resistant *Escherichia coli* was developed by inserting antibiotic resistance genes into the plasmid of bacterium. A year later, Rudolf Janish created the world's first transgenic animal by introducing foreign DNA into mice embryo. These achievements led to concerns in the scientific community about potential risks from genetic engineering.

The first genetically modified kanamycin-resistant tobacco plant (*Nicotiana plumbaginifolia*) was produced at the Washington University by Framond and his group in 1983.[18] In the same year, Jeff Schell and Marc Van Montagu of Rijks University in Ghent, Belgium, had produced transgenic tobacco plant that was resistant to kanamycin and to methotrexate, a drug used to treat cancer and rheumatoid arthritis.[19] A group of scientists of Monsanto (an Agri-based company) had developed transgenic petunia resistant to antibiotic kanamycin in the same year.[20] In 1983, another group of scientists headed by John Kemp and Timothy Hall at University of Wisconsin had inserted a bean gene into a sunflower plant. The first field trials of genetically engineered plants, transgenic tobacco to be resistant to herbicides occurred in France and the USA in 1986. In the Nineties, biotechnology was explored and moved out of the laboratory into farms and industry. In 1990 the first GM yeast(harbouring recombinant chymosin) was approved by the UK government for food making industry and in 1992, a vegetarian cheese the first food to be made from a GM ingredient went on sale in the UK.[21] China was the first country to commercialize transgenic plants, introducing a virus-resistant tobacco in 1992.[22] FlavrSavr, a tomato engineered by Calgene Company in California was the first genetically modified food crop commercialized after having been approved by the FDA.[23] It was genetically altered to have a longer shelf life by delayed fruit ripening after picking. In the same year, genetically engineered tobacco to be resistant to the herbicide bromoxynil was the first crop commercialized in Europe after approved by the European Union. In 1995, Bt-potato

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was the first pesticide producing crop approved by Environmental Protection Agency, after having been approved by the FDA. After commercialization of Bt-potato, in the same year few more transgenic crops developed by different agri-based companies received marketing approvals in US. The transgenic crops that got marketing approval were canola with modified oil composition and cotton resistant to the herbicide bromoxynil by Calgene; Bt-cotton, Bt potatoes and herbicide- immune soybeans resistant to the glyphosate known as "Round-Up- Ready" by Monsanto; Bt-corn/maize by Ciba-Geigy; virus-resistant squash by Monsanto-Asgrow; and additional delayed ripening tomatoes by DNAP Zeneca/Peto, and Monsanto.[24]

Since 1973 to 2003, top 10 genetically modified species tobacco, tomato, petunia, potato, maize, rice, wheat, barley soybean and cotton were adopted worldwide and approved for commercial cultivation. In 2009, it was reported that 25 countries including six major GMO crop growing countries USA, Brazil, Argentina, India, Canada and China adopted 11 transgenic crops for commercial cultivation. An unprecedented increase (about 100-folds) of transgenic crop cultivation was recorded from 1.7 million hectares in 1996 to 170 million hectares in 2012. In recent years, the high rate of adoption of engineered crops speaks in favor of acceptance of the transgenic technology. At present, in USA 25 GM crops have received regulatory approval to be grown commercially. In 2013, roughly 85% of corn, 91% of soybeans, and 88% of cotton grown in United States are genetically modified. In the year (2013), the National Genetically Modified Product Bio-safety Commission (KKHPRG) of Indonesia recently approved the first genetically-altered drought resistant sugarcane crop for commercial production.[24]

Perspectives on Development of Transgenic Plants

Genetically engineered plants are generated in a laboratory by altering the genetic-make-up, usually by adding one or more genes of a plant's genome. The nucleus of the plant-cell is thetarget for the new transgenic DNA. Most genetically modified plants are generated by the biolistic method (Particle gun method) or by *Agrobacterium tumefaciens* mediated transformation method. The "Gene Gun" method, also known as the "Micro-Projectile Bombardment" or "Biolistic" method is most commonly used in the species like corn and rice. In this method, DNA is bound to the tiny particles of Gold or Tungsten, which is subsequently shot into plant tissue or single plant cells, under high pressure using gun.[25] The accelerated particles arepenetrating both into the cell wall and membranes. The DNA separates from the coated metal and it integrates into the plant genome inside the nucleus. This method has been applied successfully for many crops, especially monocots, like wheat or maize, for which transformation using *Agrobacterium tumefaciens* has been less successful.[26] This technique is clean and safe. The only disadvantage of this process is that serious damage can be happened to the cellular tissue.

The next method, used for the development of genetically engineered plants, is the "Agrobacterium" method. It involves the use of soil-dwelling bacteria, known as *Agrobacterium tumefaciens*. It has the ability to infect plant cells with a piece of its DNA. The piece of DNA, that infects a plant, is integrated into a plant chromosome, through a tumor inducing plasmid (Ti plasmid). The Ti plasmid can control the plant's cellular machinery and use it to make many copies of its own bacterial DNA. The Ti plasmid is a large circular DNA particle that replicates independently of the bacterial chromosome. The importance of this plasmid is that, it contains regions of transfer DNA (t DNA), where a researcher can insert a gene, which can be transferred to a plant cell through a process known as the "floral dip". A Floral Dip involves, dipping flowering plants, into a solution of Agrobacterium carrying the gene of interest, followed by the transgenic seeds, being collected directly from the plant (Figure 1). This process is useful, in that,

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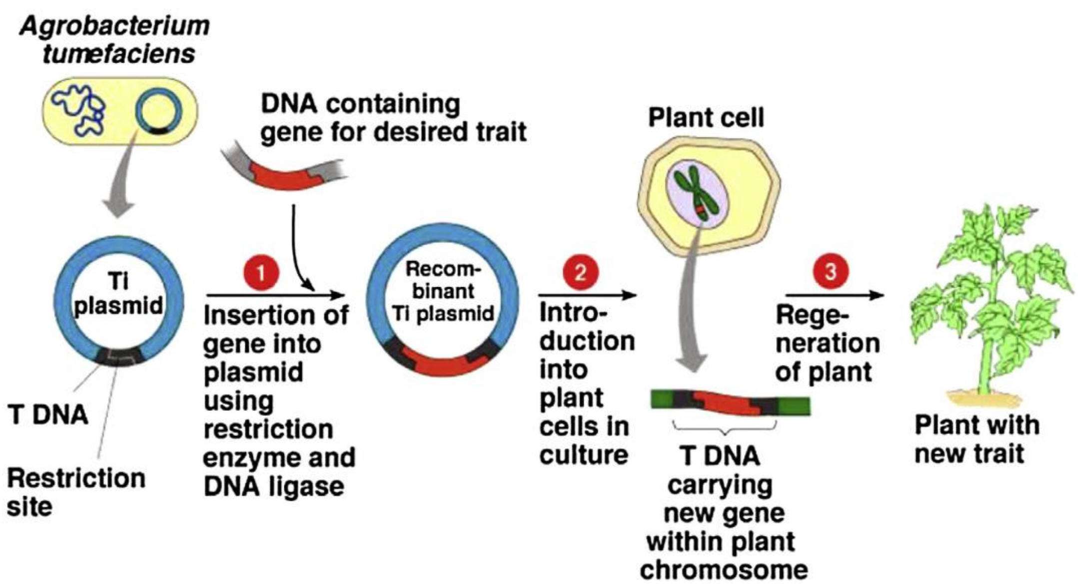
it is a natural method of transfer and therefore thought of as a more acceptable technique. In addition, "Agrobacterium" is capable of transferring large fragments of DNA very efficiently. One of the biggest limitations of Agrobacterium is that, not all-important food crops can be infected by these bacteria. This method works especially well for the dicotyledonous plants like potatoes, tomatoes and tobacco plants.[25]



Source: Jhansi Rani and Usha, (2013)[27]

Figure 1: Showing *Agrobacterium tumefaciens* mediated transformation method

Types of Transgenic Plants

Transgenic plants have genes inserted into them, deriving from other species. The inserted genes can come from species within the same kingdom (plant to plant) or between kingdoms (bacteria to plant). In many cases, the inserted DNA has to be modified slightly in order to correctly and efficiently express in the host organism. Transgenic plants are used to express proteins, like the cry toxins from *Bacillus thuringiensis*, herbicide resistant genes and antigens for vaccinations.[28]

Cisgenic plants are made up of using genes, found within the same species or a closely related one, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to crossbreed by conventional means (such as potatoes).[29]

Perspectives on Global Production

The global production status of genetically modified crops increased by 100-fold between 1996 and 2015 from 1.7 to 179.7 million ha (1996–2015). Subsequently, there was a tremendous increase in the commercialization of GM crops at a rate unsurpassed during the history of modern agriculture. Currently, USA is the world's largest producer of GM crops with 70.9 million ha (39%), 90% of which is accounted for by maize, soybean, and cotton. India has ranked fourth with 11.6 million ha of Bt cotton and has registered phenomenal growth in cotton production and topped the world with 95% resilient adoption rate. The five major global GM crops are soybean, cotton, maize, and canola. In 2015, 82% (90.7 of 111 million ha) of the soybean planted were GM soybean strains, whereas GM cotton accounted for 68% (25.1 of 37 million ha) of global cotton production.[9]

Applications of Transgenic Plants

The evaluation of the environmental impact of transgenic organisms often centres on the risks attached to them. This is justified, as any new, large-scale technology does have risks and unforeseen consequences. However, a number of arguments have suggested a positive environmental impact from largescale production of transgenic plants as follows;[30]

Reduced environmental impact from pesticides: Herbicides and pesticides have potential hazards for environmental pollutions, whereas, transgenic crops may decrease the use of environmentally harmful chemicals to control weeds and pests.[30] For example, reduced frequency of treatments can bring a net decrease in pesticide pollution if paralleled with a decrease in the total amount of pesticide and herbicide used. Conflicting claims have been made

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about the effect of herbicide tolerant crops in the U.S.A.[31] In the absence of published documentation where the assumptions and the validity of the arguments can be checked, no conclusions can be drawn.[30]

Increased yield: If crop yields increased, less cultivated area would be needed to produce the total amount of food required by people. This could result in a lower pressure on land not yet under cultivation and could allow more land to be left under protection. The potential environmental benefits of this type may be greatest in developing countries where most of the agricultural production increase was due to new areas taken into cultivation.[32]

Soil conservation: Herbicide-tolerant crops may allow farmers to abandon the use of soil-incorporated pre-emergent herbicides. This shift to post emergent weed control may increase the no-till and conservation till age practices, decreasing soil erosion, water loss, and increasing soil organic matter.[33]

Phytoremediation: Emphasis has been given for in-situ remediation of soil and water pollution by transgenic plants and micro-organisms. Transgenic plants can sequester heavy metals from soils[34] or detoxify pollutants.[35]This has not yet been used widely, so its environmental impact has not been studied.

GM Technology has been used to produce a variety of crop plants to date. As the global population continues to expand, food remains a scare resource. Genetically engineered foods offer significant benefits by improving production yield, lowering transportation costs and enhancing the nutritional content. Developments, resulting in commercially produced varieties in countries such as USA and Canada, have centered on conferring resistance to insect, pests or viruses and producing tolerance to specific herbicides. While these traits had benefits for the farmers, it has been difficult for the consumers to see any benefit other than these. In limited cases, a decreased price owing to reduced cost and increased ease of production.[36]

Herbicide resistant plants: Plants that can tolerate herbicides are called Herbicide Resistant Plants. Glyphosate is an active ingredient of many broad-spectrum herbicides. Glyphosate resistant transgenic tomato, potato, tobacco, cotton etc… are developed by transferring aro A gene into a glyphosate EPSP synthetase from *Salmonellatyphimurium* and *E. coli* Sulphonylurea resistant tobacco plants are produced by transforming the mutant ALS (acetolactate synthetase) gene from Arabidopsis. QB protein of photo system II from mutant *Amaranthus hybrids* is transferred into tobacco and other crops to produce atrazine resistant transgenic plants.[27]

Insect resistant plants:Bacillus thuringiensis is a bacterium that is pathogenic for a number of insect pests. Its lethal effect is mediated by a protein toxin it produces. Through recombinant DNA methods, the toxin gene can be introduced directly into the genome of the plant, where it is expressed and provides protection against insect pests of the plant.[27]

Virus resistant plants: TMV resistant tobacco and tomato plants are produced by introducing viral coat proteins. Other viral resistant transgenic plants are as follows; (i) Potato virus resistant potato plants, (ii) RSV resistant rice, (iii) YMV resistant black gram and (iv) YMV resistant green gram.[27]

Nutritional benefits: Vitamin A deficiency causes half a million children to become partially or totally blind each year.[37-39] Milled rice is the staple food for a large fraction of the World's human population. Traditional breeding methods have been unsuccessful in producing crops, containing a high concentration of vitamin A. Researchers have introduced three genes into rice: two from daffodils and one from a microorganism. The transgenic rice exhibits an

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increased production of beta-carotene as a precursor to vitamin A and the seed is yellow in color.[40] Such yellow, or golden, rice may be a useful tool to treat the problem of vitamin A deficiency in young children living in the tropics.

Therapeutic proteins from transgenic plants: Proteins of therapeutic importance, like those used in the treatment, diagnosis of human diseases can be produced in plants, using recombinant DNA technology. Scaling-up of these transgenic plants to fields, results in industrial production of proteins. The area of research combining molecular Biotechnology and Agriculture is called Molecular farming or pharming. The proteins produced in transgenic plants for therapeutic use, are of following three types; (i) antibodies, (ii) proteins and (iii) vaccines. Antibodies directed against dental caries, rheumatoid arthritis, cholera, E. coli diarrhea, malaria, certain cancers, HIV, rhinovirus, influenza, hepatitis B virus andherpes simplex virus are known to be produced in transgenicplants. Vaccines against infectious diseases of the gastro intestinaltract have been produced in plants like potato andbananas.[41]Another appropriate target would be cerealgrains. An anti-cancer antibody has recently expressed in riceand wheat seed that recognizes cells of lung, breast and coloncancer and hence could be useful in both diagnosis andtherapy in the future.[42]

Conclusion

In conclusion, farmers receive significant multiple benefits from GM foods as commodity crops in terms of social, economic, and health benefits. Transgenic plants should be promoted in developing countries because their practical application may strengthen their economies. Additionally, researchers hope to be able to deliver vaccines and medications in GM foods in the future, making it easier for people in developing nations to access medication. However, effect of transgenic crops on the ecosystems should be monitored regularly to avoid the potential risk factors.

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