Journal of Cell and Tissue Research Vol. 17(2) 6185-6196 (2017)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Review Article

RECENT ADVANCES IN TRANSGENIC CROPS: A REVIEW

PRASAD, B. D.,[1]? RANJAN, T.,[2] SAHNI, S.[3] AND SHARMA, V.[4]

[1]Department of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur 813210 (Bihar); [2]Department of Basic Science & Humanities-Genetics, Bihar Agricultural University, Sabour, Bhagalpur 813210 (Bihar); [3]Department of Plant Pathology, T.C.A., Dholi, Dr. Rajendra Prasad Central Agricultural University, Pusa 843121 (Bihar); [4]DOS in Biotechnology, University of Mysore 570006 (Karnataka) E. mail: dev.bishnu@gmail.com

Received: July 5; 2017; Accepted: July 20, 2017

Abstract: In recent years there has been a tremendous increase in the application of transgenic crop for the production and development of variety of commercially valuable biological molecules for the purpose of human and animal healthcare. Intriguingly, recent advancement made transgenic crops (as a whole and cell culture systems) as a melting pot or biological factories for the production of large-scale quantities of antibodies, antigens and/or vaccine epitopes, metabolic enzymes, hormones, neuropeptides and a variety of biologically active complexes and secondary metabolites for direct use as therapeutic agents or diagnostic tools in the medical healthcare industry. Transgenic crop researches rely on the methods of transformation either by indirect Agrobacterium mediated or direct gene transfer. The "first generation "of transgenic crops were aimed at improving traits involving single genes. Now we are on the verge of a new step in crop modification, fueled by the rate at which new genes (important for plant growth and development metabolism and stress tolerance) characterized. Reinforcement of resistance against insect-pests and pathogens attack using genetic engineering has proven to be an effective strategy to develop resistant crop plants and that could offer a remedy, allowing more precise targeting of pest and disease management. Transgenic technology has been pivotal in the full spectrum of these new developments, from gene identification to an improved understanding of their regulation, as well as genetic transformation involving more complex transfers of many genes simultaneously. As the products of genetically modified crops or transgenic crops make their way from concept to commercialization, the associated risks and acceptance by the public sector has been become a major challenge. In this paper, we revisit the recent advances made in the genetically modified crops for their improvement and protection.

Key words: Transgenic crop

INTRODUCTION

Genetic modification of crops has empowered us to modify plants in a novel ways and has the great potential to combat important problems in the field of agriculture. Genetically Modified (GM) plants or transgenic plants are generally produced by addition of gene(s) obtained from same or different species or chemically synthesized leading to modification of the plant's/organism's genome with the help of recombinant DNA (rDNA) techniques. The idea behind developing transgenic crops is to introduce a trait that does not naturally occur in the plant to help improve, and protect the crop. Genetic engineering within food crops is done to create resistant to disease, pest, environmental stress condition, reduction of

spoilage, resistant to chemical treatment (such as herbicides), and improving the nutrient profiling. The non-food crops are also being utilized for the production of pharmaceutical agents, biofuel and bioremediation as well [1-3]. Development of transgenic crops research depends on the availability of procedures for plant transformation. Two types of method for plant transformation are exist till date; (I) use of *Agrobacterium* as a biological vector for foreign gene transfer, and (II) direct gene transfer techniques, in which DNA is introduced into cells by the use of physical, electrical or chemical means [4]. Using this procedures thousands of transgenic crops have been developed experimentally or field tested, while few of them are currently cultivated worldwide, offering the potential increasing and improving food production capacity while limiting the use of agrochemicals and protect the environment. *Agrobacterium* can be used to transform a wide range of plants, but there are still many species remains which are of great interest for basic or applied research in which *Agrobacterium* mediated transformation is not efficient [4-5]. Recent advancement indicates that these host-range limitations can be overcome by developing specific plant cell culture procedures and defining inoculation and co-cultivation conditions. Some important non-host species such as maize and rice have now been stably transformed by *Agrobacterium* [6]. Many new transgenic varieties have been produced those are resistant to insects, herbicides, pathogens or express novel characters that improve product quality, yield and agronomic traits. The new opportunities to modify plants in a novel ways with genetic modification present new responsibilities for safe use to avoid adverse effects on human health and the environment [6]. For the production and launching of new transgenic variety to the market, risk assessment studies have become an integral part. Several countries have opted different approaches in the biosafety assessment.

The month of January marks the 32th anniversary of the first successful introduction of a foreign gene into a plant using *Agrobacterium tumefaciens* [4]. *A. tumefaciens*, a soil born, gram negative bacterium described as a "natural genetic engineer", transfer its own genes (T-DNA) into host plant cells. This pathogenic bacterium was now converted into a pack mule, to carry new, foreign genes into plant cells, and this became the most common means of producing genetically engineered plants. Transgenic crops **grab media** headlines when first genetically engineered crop "Flavr Savr" (pronounced "flavor saver"), a genetically modified tomato, approved for commercially cultivation for human consumption in 1994. With this the era of transgenic crop cultivation begins and till than biotech crops have been successfully grown in accumulated hectarage of 1.78 billion hectares (4.4 billion acres). In 2014, the global area of biotech crops continued to increase for the 19th year at a sustained growth rate of 3 to 4% or 6.3 million hectares (~16 million acres), reaching 181.5 million hectares or 448 million acres. Biotech crops have set a precedent in that the biotech area has grown impressively every single year for the past 19 years, with a remarkable 100-fold increase since the commercialization began in 1996. Thus, biotech crops are considered as the fastest adopted crop technology in the history of modern agriculture. In 2014, for the third time, more than half (53%) of the global biotech crop area of 181.5 million hectares, equivalent to 96.2 million hectares, was grown in 20 developing countries. Unlike 2013, year-to-year growth was higher in the industrial countries at 4.2 million hectares (5%) than in developing countries at 2.1 million hectares equivalent to a 2% growth; this was principally due to higher growth in the US (soybean) and Canada (canola) in 2014. Thus, whereas year-to-year growth was significantly faster in industrial countries in 2014, developing countries maintained a larger share of global biotech crops at 53% compared with only 47% for industrial countries. The number of countries growing GM crops has increased to 29 in recent years. This suggests that the GM crops are the fastest adopted technology in the field of agriculture [7].

To feed the several billion people living on this planet, the production of high-quality food must increase with reduced inputs, but this accomplishment will be particularly challenging in the face of global environmental change. Plant breeders need to focus on traits with the greatest potential to increase yield. Hence, new technologies must be developed to accelerate breeding through improving genotyping and phenotyping methods and by increasing the available genetic diversity in breeding germplasm. Most of the gain will come from delivering these technologies in developing countries, but the technologies will have to be economically accessible and readily disseminated. Crop improvement through breeding brings immense value relative to investment and offers an effective approach to improving food

security. However, to meet the recent Declaration of the World Summit on Food Security target of 70% more food by 2050, an average annual increase in production of 44 million metric tons per year is required. Particularly challenging for society will be changes in weather patterns that will require alterations in farming practices and infrastructure; for example, water storage and transport networks. The likely impacts on global food production are many because one-third of the world's food is produced on irrigated land. Along with agronomic- and management-based approaches to improving food production, improvements in a crop's ability to maintain yields with lower water supply and quality will be critical [7]. By and large, we need to increase the tolerance of crops to biotic and abiotic stress conditions by several folds. Modern tools of plant biotechnology can complement conventional plant breeding in an economically useful way to genetically improve crop plants. In genetically modified (GM) crop plants, their genome is engineered using tools of genetic engineering such as recombinant DNA technology, which is complemented by our knowledge of molecular biology [8]. In this approach, different DNA fragments from various useful sources are put together to create a new molecule that is introduced into the plant genome for desired purposes. Thus, essentially *transgenic plants* are those plants containing DNA from other organisms. Remarkably, while developing transgenic plants the genetic engineer enjoys advantage of cross-species gene transfer and considerable reduction in time toward generating an improved transgenic line for a specific crop plant. In the distant and recent past we have relied on domestication of crop plants, development of hybrid seeds and experienced "*green revolution*" through advances in plant breeding technologies. In recent years, we have been witnessing a "*gene revolution*" that is making remarkable advance in the field of plant biotechnology [9]. Genetic engineering involves cloning of desired genes, development of designer gene constructs and transfer of transgenes to the organism concerned. Specific changes are introduced in the genome of crop plants using the tools of genetic engineering. Over last three decades, a large number of transgenic plants have been developed across different classes of crops with various improved agronomic characteristics [10]. The main focus has been development of transgenic crop plants for enhanced resistance to bacterial diseases, fungal diseases, virus, nematode, insect pests etc. and tolerance to drought, salinity, flooding, heavy metals etc [9-13]. The first GM crop produced was an antibiotic-resistant tobacco plant in 1982 and the first field trials were conducted in France and the USA in 1986, when tobacco plants were engineered for herbicide resistance. Plant Genetic Systems (Belgium), founded by Montagu and Schell, was the first company to produce genetically engineer insect-resistant (tobacco) plants by incorporating genes that produced insecticidal proteins from Bacillus thuringiensis (Bt) in 1987 [14,15]. China was the first country to allow commercialized transgenic virus-resistant tobacco plant. But first time GM crop was approved for the sale in the U.S., in 1994, which was the Flavr Savr tomato. It had a longer shelf life, because it took longer to soften after ripening. In 1994, Europe approved tobacco engineered which was resistant to the herbicide bromoxynil. In 1995, Bt Potato, Bt maize, glyphosate-resistant soybeans, virus-resistant squash, and additional delayed ripening tomatoes were approved. In 2000, Vitamin A-enriched golden rice, was the first food with increased nutrient value [1,16]. Plants engineered to tolerate non-biological stressors such as drought, frost, high soil salinity, and nitrogen starvation are in the process of development. In 2011, Monsanto's Drought Gard maize became the first drought-resistant GM crop to receive US marketing approval. In 2012, the FDA approved the first plant-produced pharmaceutical, a treatment for Gaucher's Disease [17].

Till date several plants including tobacco plants have been modified to produce therapeutic antibodies. In 2005, about 13% of the Zucchini (a form of squash) grown in the US was genetically modified to resist three viruses. In 2011, the potato was made resistant to late blight by adding resistant genes *blb1* and *blb2* that originate from the Mexican wild potato *Solanum bulbocastanum* [18]. In 2013, the USDA approved the import of a GM pineapple that is pink in color and that "overexpresses" a gene derived from tangerines and suppress other genes, increasing production of lycopene. In 2013, Robert Fraley, Marc Van Montagu and Mary-Dell Chilton were awarded the World Food Prize for improving the "quality, quantity or availability" of food in the world for applying genetic engineering technology in the crops. In 2014, the USDA approved a genetically modified potato developed by J.R. Simplot Company that contained ten genetic modifications that prevent bruising and produce less acrylamide when fried. The modifications eliminate specific proteins from the potatoes, via RNA interference, rather than

introducing novel proteins [19]. In February 2015, Arctic Apples were approved by the USDA and becoming the first genetically modified apple approved for sale in the US. Gene silencing was used to reduce the expression of polyphenol oxidase (PPO), thus preventing the fruit from browning [1,20]. Cereals such as Corn basically used for the food and ethanol production has been genetically modified to tolerate various herbicides by expressing a protein from *Bacillus thuringiensis* (*Bt*) that kills certain insects. In 2015, 81% of corn acreage contained the Bt trait and 89% of corn acreage contained the glyphosate-tolerant trait in US. Corn can be processed into grits, meal and flour as an ingredient in pancakes, muffins, doughnuts, breadings and batters, baby foods, meat products, cereals and some fermented products as well. Corn-based masa flour and masa dough are used in the production of taco shells, corn chips and tortillas [1]. However, although many genetically modified (GM) crop plants have been developed, only a few of them have made their way to the field. On the contrary, the land area under GM crop cultivation has increased steadily over last decade though it has mainly remained restricted to the countries such as USA, Argentina, Brazil, Canada, India, China and so on. Some of the GM crop plants that are being grown in the field are cotton, corn, soybean, canola, sugarbeet, papaya, alfalfa, brinjal etc. In total 29 countries allow growing of GM crops with 181.48 mha area.

Different methods of gene transfer in plants: The most widely used method for the introduction of new genes into plants is based on the natural or direct DNA transfer capacity of *Agrobacterium tumefaciens*. In nature this soil bacterium causes tumor formation (called crown gall) on a large number of dicotyledonous plant species. During this infection a part of the Ti-plasmid of *Agrobacterium*, called T-DNA, is transferred and integrated into the plant genome [21]. This fascinating natural capacity made us use this bacterium as a natural vector of foreign genes (inserted into the Ti-plasmid) into plant chromosomes. *Agrobacterium*-based and direct gene transfer techniques were developed in parallel, but the former is today the most widely-used method because of its simplicity and efficiency in many plants, although it still suffers limitations in terms of the range of species which are amenable to transformation [4]. These limitations are due to the natural host range of *Agrobacterium*, which generally infects herbaceous dicotyledonous species most efficiently and is less effective on monocotyledonous and woody species [21].

The development of novel direct gene transfer methodology, by-passing limitations imposed by *Agrobacterium*-host specificity and cell culture constraints, has allowed the engineering of almost all major crops, including formerly recalcitrant cereals, legumes and woody species [4]. Direct gene transfer transformation methods are species and genotype-independent in terms of DNA delivery, but their efficiency is influenced by the type of target cell, and their utility for the production of transgenic plants in most cases depends on the ease of regeneration from the targeted cells, as most methods operate on cells cultured *in vitro* [22]. As direct gene transfer referred methods such as particle bombardment, DNA uptake into protoplasts, treatment of protoplasts with DNA in the presence of polyvalent cations, fusion of protoplasts with bacterial spheroplasts, fusion of protoplasts with liposomes containing foreign DNA, electroporation-induced DNA uptake into intact cells and tissues, silicon carbide fiber-induced DNA uptake, ultrasound-induced DNA uptake, microinjection of tissues and cells, electrophoretic DNA transfer, exogenous DNA application and imbibition, macroinjection of DNA [22]. The major achievements of transgenic plant technology up to now concern tolerance to insect or disease pests, herbicide tolerance, and improved product quality. A description of the major categories of modified traits with characteristic examples will follow.

Engineered crops resistance to insect pests using Bt-toxin: In the recent year, chemical control method is generally preferred over continuous plant breeding efforts for the management of insect pests. Many fascinating new genetic methods for insect control are being used these days, which could substantially reduce expenditures and crop losses, and to be precise, these methods are less detrimental to the environment. A very successful molecular approach to engineering resistance has involved generating chimeric plants with the capability of synthesizing antimicrobial or insecticidal products. These products are usually constitutively produced in plants. This means that these insecticidal genes are put up under the control of strong constitutive promoters. In many cases, an inducer or activator is necessary for the gene expression in order to activate synthesis of these engineered chemicals if the presence of a pest is detected (e.g., the tetracycline-

inducible promoter system). One of the drawbacks of this system is requirement of large quantities of tetracycline for induction, which makes this system not to be ideal. Therefore, alternative inducers are currently being developed by the researchers.

Btδ-endotoxins: *Bacillus thuringiensis* (Bt), a Gram-positive soil bacterium, produces Btδ-endotoxins, crystalline inclusions during sporulation. These inclusions contain several insecticidal proteins out of which over 100 different Bt toxins have been identified till date. The Btδ-endotoxins are processed inside the insect midgut to form the active form of toxin. Numerous plant species have now been transformed with Btδ--endotoxin, a bacterial gene expressing the insecticidal proteins, making the plants tissues toxic to several insect pests. The Btδ-endotoxin gene was cloned in 1981 for the first time and the source of gene was from *B. thuringiensis*. Moreover, a research article on transgenic plants protected from insects by δ-endotoxins was published in 1987 [23]. Commercial seeds are available for several important crops including corn, potato, and cotton, expressing different synthetic Bt genes that show significant protection against the European corn borer, colorado potato beetle, cotton bollworm, and pink bollworm infestations. Btδ-endotoxin expression is currently under development stage in crops such as alfalfa, apples, cranberry, eggplant, rice, and other plant species. One of the major challenges for scientific community is the durability and stability of produced Bt δ-endotoxins, due to the fact that certain pests have shown resistance to some of the toxins [23].

Apart from *Bt* gene, several other insecticidal proteins are derived from plants which include chitinases, peroxidases, β-amylase inhibitors, proteinase inhibitors, trypsin inhibitors, and lectins. These proteins have also very significant effect on pest resistivity by the crops harboring these genes. Transgenic plants expressing these compounds have been generated and evaluated for control of various pests worldwide. Recently, transgenic tobacco has been used for expressing proteinase inhibitors and peroxidase as well, for control of *Manducasexta* larvae and *Helicoverpa zea*. Interestingly, transgenic pea seedlings expressing alpha-amylase inhibitor showed significant resistance to bruchid beetles. *Streptomyces*-derived cholesterol oxidase proteins have also recently been reported to have insecticidal activity worldwide against the cotton boll weevil, which is very difficult to control using conventional pesticide sprays. These compounds are being widely expressed in transgenic tobacco cells and may prove very useful alternative in the coming future [24].

A number transgenic crop plants have been developed for increased resistance to variety of insect pests using variety of strategies (Table 1). One of the most skyrocketing achievements in plant biotechnology is development of insect resistant crops expressing crystal proteins from *Bacillus thuringiensis* (*Bt*). *B. thuringiensis* is a gram positive bacterium that produces proteinaceous crystalline (Cry) inclusion bodies during sporulation. It also produces cytotoxins that synergize the activity of Cry toxins [25]. It is known that the Bt crystal proteins (δ-endotoxin) are toxic to lepidopterans, dipterans, and coleopterans and at the same time it is non-toxic to humans and animals [26]. This protein has been used as a pesticide spray for many years. Cultivation of these transgenic plants should help reduce the use of chemical pesticides in cotton production, as well as in the production of many other crops, which could be engineered to contain the *Bacillus thuringiensis* gene.

Engineered crops resistance to fungal pathogens using genes for chitinases and glucanases: Various transgenic crop plants have been developed for enhanced resistance to number of fungal pathogens using variety of strategies (Table 1). In recent years, several laboratories have transformed plants with genes encoding β-1,3-glucanase and chitinase in order to develop transgenic crops with enhanced resistance to fungal diseases. Chitinase appears to have been used probably most frequently to obtain transgenics in various crops for effective control of fungal pathogens. The genes for chitinase from varied sources have been used to generate transgenics in grapevine [27], rice [28] and peanut [29].

Engineered crops resistance to viral disease: Viruses cause many economically important plant diseases. For example, the *Beet necrotic yellow vein virus* (BNYVV) causes sugar beets to have smaller, hairier roots, reducing yields by up to 50%. The spread of most viruses is very difficult to control. Once infection sets in, no chemical treatment methods are available. Losses are usually very high and require longer rotation intervals and modified cropping systems. This translates into considerable losses. Viruses are often transmitted from plant to plant by

Table 1: Recent developments of transgenic plants resistant to insect pests and diseases (bacterial, fungal and viral).

Sl. No.	Transgene	Source	Crop & cultivar	Resistance against
Insect pests resistance				
1.	*amiR-24*	*B. thuringiensis*	Tobacco	Cotton bollworm
2.	*Bt (?-endotoxin gene)*	*B. thuringiensis*	Rice	Striped stem borer
3.	*HaAK*	*Arabidopsis sp.*	*Arabidopsis sp.*	Cotton bollworm
4.	*cry3A*	*B. thuringiensis*	Potato	Colorado potato beetle
5.	*cry1Ia8*	*B. thuringiensis (Btc008)*	Cabbage	Diamondback moth
6.	*HaHR3*	Cotton bollworm *(H. armigera)*	Tobacco *(N. tabacum)*	Cotton bollworm
7.	*EcR*	Cotton bollworm *(H. armigera)*	Tobacco *(N. tabacum)*	Cotton bollworm
8.	*Cry1Ab*	*B. thuringiensis*	Rice	Lepidopteron Leaf folder and stem borer
Fungal pathogens resistance				
1.	*Chitinase*	*Streptomyces griseus*	*Brassica juncea*	Wide range of Fungal pathogens
2.	*HaGLP1* (Germin like proteins)	Sunflower (*Helianthus annuus*)	*Arabidopsis thaliana*	*Sclerotinia sclerotiorum and Rhizoctonia solani*
3.	*Chi11*	Rice	Finger millet (*Eleusine coracana L.*)	Leaf blast
Bacterial pathogens				
1.	*hRPN*	*Erwinia amylovora*	Pears (*Pyrus communis cv. 'Passe Crassane'*)	*Erwinia amylovora*
2.	*FALL39* (precursor for the antimicrobial peptide LL-37)	*Homo sapiens*	Chinese cabbage (*Brassica rapa* cv. Osome)	*Psanthomonascarotovorum*
3.	*Bs2*	Pepper	Tomato (*Solanum lycopersicum*)	*Xanthomonas*
Viral pathogens				
1.	Coat protein gene, V2 gene and replication-associated gene	*Tomato yellow leaf curl virus*-Oman (TYLCV-OM)	*Tomato (Solanumlycopersicum* L.)	TYLCV-OM
2.	*pC5, pC6*	RGSV	Japonica rice	Rice grassy stunt virus (RGSV)
3.	*CP*	Tobacco Streak Virus	Sunflower (*Helianthus annuus* L.)	Tobacco Streak Virus
4.	*Rep* (Replication Initiation protein)	*Banana bunchy top virus* (BBTV)	Banana (*Musa* spp.)	Banana bunchy top virus (BBTV)
Plant pathogenic nematode				
1.	*16D10*	Conserved Root-Knot Nematode (RKN) gene *16D10*	Wine grape (*V. vinifera*cv. Chardonnay)	Root knot nematode
2.	CCII (Cystatin)	Maize kernel	Plantain (*Musa AAB* cv. GonjaManjaya)	*Radopholussimilis, Helicotylenchusmulticinctus*

Table 2: Range of plant viruses used for silencing of target genes and their hosts with targeted genes

Virus	Silencing Host	Gene Silenced
Tobacco mosaic virus	*N. benthamiana N. tabacum*	*pds*
Potato virus X	*N. benthamiana Arabidopsis*	*pds*
Tobacco rattle virus	*N. benthamiana,* Tomato, Solanum, Chili pepper	*Rar1, EDS1, NPR1/ NIMI, pds, rbcS*
Barley stripe mosaic virus	Barley	*Pds, Lr21, Rar1, Sgt I, Hsp90*
Bean pod mottle virus	Glycine max	*pds*
Cabbage leaf curl virus	Arabidopsis	*CH42, pds*
Pea early browning virus	*Pisum sativum*	*Pspds, uni, kor, pds*
Tomato yellow leaf curl china virus	*N. benthamiana*	*Pcna, pds, su, gfp*
African cassava mosaic virus	*N. benthamiana*	*Pds, su, cyp79d2*

insects. Insecticides are sometimes used to control viral infections, but success is very limited. The most effective ways of managing viruses are cultural controls (e.g., removing diseased plants) and using resistant cultivars. Although conventional methods of breeding have been able to provide some virus-resistant or tolerant cultivars, they are not available for most crops. In some cases, biotechnology can be used to make virus-resistant crops [30]. The most common way of doing this is by giving a plant a viral gene encoding the virus' "coat protein" (Table 2). The plant can then produce this viral protein before the virus infects the plant. If the virus arrives, it is not able to reproduce. The explanation for this is called cosuppression or virus-induced gene silencing (VIGS). The plant has ways of knowing that the viral coat protein should not be produced, and it has ways

of eventually shutting down the protein's expression. When the virus tries to infect the plant, the production of its essential coat protein is already blocked. All genetically modified virus resistant plants on the market (e.g., papayas and squash) have coat protein mediated resistance. It may also be possible to confer resistance by taking a resistance gene naturally found in one plant and then transferring it to an important crop [30].

Virus-induced gene silencing (VIGS) is a virus vector technology that exploits an RNA-mediated antiviral defense mechanism. In plants infected with unmodified viruses, the mechanism is specifically targeted against the viral genome. However, with virus vectors carrying inserts derived from host genes the process can be additionally targeted against the corresponding mRNAs. VIGS has been used widely in plants for analysis of gene function and has been adapted for high-throughput functional genomics. Until now, most applications of VIGS have been studied in *Nicotiana benthamiana*. However, new vector systems and methods are being developed that could be used in other plants, including Arabidopsis [31]. VIGS also helps in the identification of genes required for disease resistance in plants. These methods and the underlying general principles also apply when VIGS is used in the analysis of other aspects of plant biology (Fig.1). Table 2 summarizes the range of plant viruses with their suitable hosts used in RNA interference technology till date (Table 2). When a plant virus infects a host cell, it activates an RNA-based defense that is targeted against the viral genome. The double-stranded RNA (dsRNA) in virus-infected cells is thought to be the replication intermediate that causes the small interfering RNA/ribonuclease (siRNA/RNase) complex to target the viral single-stranded RNA (ssRNA). In the initially infected cell, the viral ssRNA would not be a target of the siRNA/RNase complex because this replication intermediate would not have accumulated to a high level. However, in the later stages of the infection, as the rate of viral RNA replication increases, the viral dsRNA and siRNA would become more abundant. Eventually, the viral ssRNA would be targeted intensively and virus accumulation would slow down [32]. Many plant viruses encode proteins that are suppressors of this RNA silencing process. These suppressor proteins would not be produced until after the virus had started to replicate in the infected cell so they would not cause complete suppression of the RNA-based defense mechanism. However, these proteins would influence the final steady-state level of virus accumulation. Strong suppressors would allow virus accumulation to be prolonged and at a high level. Conversely, if a virus accumulates at a low level, it could be due to weak suppressor activity. The dsRNA replication intermediate would be processed so that the siRNA in the infected cell would correspond to parts of the viral vector genome, including any non-viral insert. Thus, if the insert is from a host gene, the siRNAs would target the RNase complex to the corresponding host mRNA and the symptoms in the infected plant would reflect the loss of the function in the encoded protein [33].

There are several examples that strongly support this approach to suppression of gene expression. Thus, when tobacco mosaic virus (TMV) or potato virus X (PVX) vectors were modified to carry inserts from the plant phytoene desaturase gene (*pds*) the photobleaching symptoms on the infected plant reflected the absence of photoprotective carotenoid pigments that require phytoene desaturase. Similarly, when the virus carried inserts of a chlorophyll biosynthetic enzyme, there were chlorotic symptoms and, with a cellulose synthase insert, the infected plant had modified cell walls [34]. Genes other than those encoding metabolic enzymes can also be targeted by VIGS. For example, if the viral insert corresponded to genes required for disease resistance, the plant exhibited enhanced pathogen susceptibility. In one such example, the insert in a tobacco rattle virus (TRV) vector was from a gene (EDS1) that is required for N-mediated resistance to TMV. The virus vector-infected N-genotype plant exhibited compromised TMV resistance. The symptoms of a TRV vector carrying a *leafy* insert demonstrate how VIGS can be used to target genes that regulate development. *Leafy* is a gene required for flower development. Loss-of-function *leafy* mutants produce modified flowers that are phenocopied in the TRV-leafy infected plants. Similarly, the effects of tomato golden mosaic virus vectors carrying parts of the gene for a cofactor of DNA polymerase illustrate how VIGS can be used to target essential genes. The plants infected with this geminivirus vector were suppressed for division growth in and around meristematic zones of the shoot [35].

To exploit the ability to knock down, in essence, any gene of interest, RNAi via siRNAs has generated a great deal of interest in both basic and applied biology.

Table 3: Recent developments of transgenic plants resistant to abiotic stresses.

Sl. No.	Transgene	Source of transgene	Crop & Cultivar
1.	BADH, betaine aldehyde dehydrogenase gene	*Atriplex micrantha*	Maize elite inbred lines, Zheng58 and Qi319
2.	AcPIP2, Plasma membrane Aquaporin gene	*Atriplex canescens*	*Nicotiana benthamiana„ Arabidopsis thaliana* Col-1
3.	OCPI2, chymotrypsin protease inhibitor	*Oryza sativa* PB-1	*Arabidopsis thaliana* Columbia
4.	WT-PhyA, S599A-PhyA	*Avena sativa*	*Zoysia grass* (Zoysia Japonica Steud.), Creeping bentgrass (Agrostisstolonifera L.)
5.	MusaPIP2;6, aquaporin gene	Banana, cv. Karibale Monthan	Banana, cv. Karibale Monthan
6.	SOS2, salt overly sensitive gene	*Populus trichocarpa*	Aspen hybrid clone Shanxin Yang (*Populus davidiana* X *Populus bolleana*)
7.	LCY-ε, Lycopene ε-cyclase	*Ipomoea batatas* cv. Yulmi Wild type	*Ipomoea batatas*
8.	AtNHX1, Na+/H+ antiporter gene	*Arabidopsis thaliana*	*Zea mays*
9.	JcDREB, Stress responsive DNA binding Transcription Factor	*Jatropha curcas*	*Arabidopsis thaliana*
Drought stress tolerance			
1.	BdWRKY36 (WRKY transcription factor)	*Brachypodium distachyon*	*Nicotiana tabaccum*
2.	AtEDT1/HDG11 (Homodomain-leucine zipper transcription factor Enhanced Drought Tolerance/HOMEODOMAIN GLABROUS11)	*Arabidopsis thaliana* Col-0	Oryza sativa japonica
3.	TaMYB30-B (MYB type gene)	Wheat of different ploidy levels	*Arabidopsis thaliana*
4.	OtsA, OtsB (Trehalose -6-P synthase, trehalose-6-P phosphatase)	*E. coli*	Rice Pusa Basmati-1 (PB-1)
5.	TaWRKY19 (WRKY type Transcription factor)	*Triticum aestivum*	*Arabidopsis thaliana*
Cold stress tolerance			
1.	CsTK (Transketolase)	cDNA library	*Cucumis sativa* L. cv Jinyou 3
2.	DREB1B, (dehydration-responsive element binding factor 1)	Arabidopsis sp.	*Solanum tuberosum* L.
3.	TaWRKY19	*Triticum aestivum* L. cultivar Xifeng 20	Arabidopsis ecotype Columbia plants (Col-0)

Increasing number of large-scale RNAi screens are designed to identify the important genes in various biological pathways. Because disease processes also depend on the combined activity of multiple genes, it is expected that turning off the activity of a gene with specific siRNA could produce a therapeutic benefit to humanity. Based on the siRNAs-mediated RNA silencing (RNAi) mechanism, several transgenic plants has been designed to trigger RNA silencing by targeting pathogen genomes. Diverse targeting approaches have been developed based on the difference in precursor RNA for siRNA production, including sense/antisense RNA, small/long hairpin RNA, and artificial miRNA precursors. Virologists have designed many transgenic plants expressing viral coat protein (CP), movement protein (MP), and replication-associated proteins, showing resistance against infection by the homologous virus. This type of pathogen-derived resistance (PDR) has been reported in diverse viruses including tobamo-, potex-, cucumo-, tobra-, carla-, poty-, and Alfalfa mosaic virus groups as well as the luteovirus group [10, 36]. Transgene RNA silencing-mediated resistance is a process that is highly associated with the accumulation of viral transgene-derived siRNAs. One of the drawbacks of the sense/antisense transgene approach is that the resistance is unstable, and the mechanism often results in delayed resistance or low efficacy/resistance. This may be due to the low accumulations of transgene-derived siRNA in post-transcriptional gene silencing (PTGS) due to defense mechanism encoded by plants. Moreover,

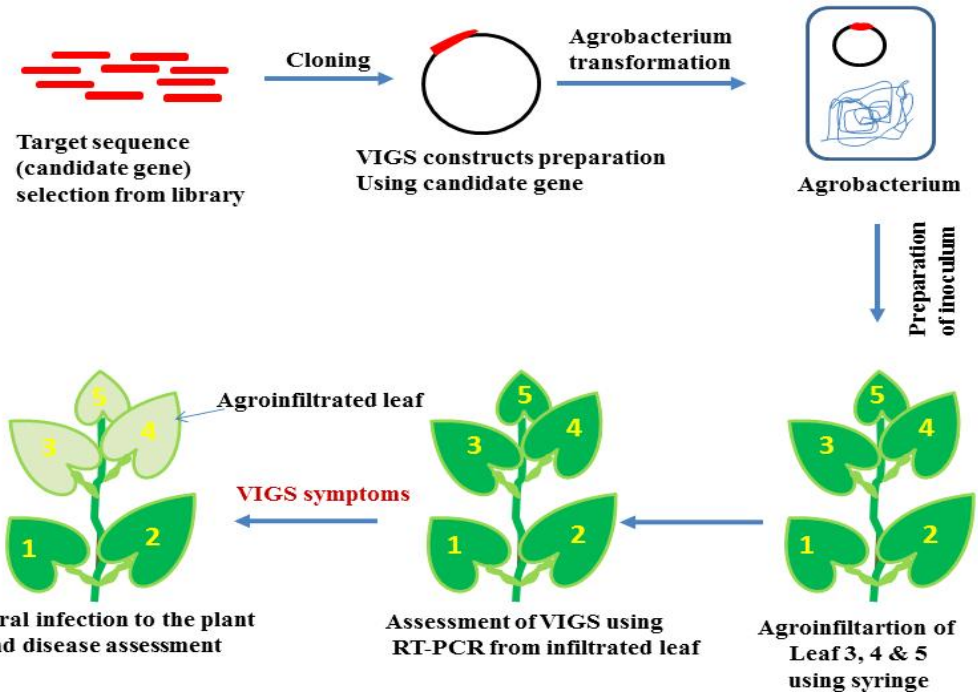

Fig. 1: Steps of virus-induced gene silencing (VIGS). VIGS starts by the cloning of the target gene fragment (200–300 bp) into a virus infectious cDNA, which is in a binary vector under the control of the CaMV 35S promoter. The recombinant virus construct is then transformed into *Agrobacterium* (*Agrobacterium tumefaciens*) for *Agrobacterium* mediated virus infection. VIGS will target to the virus carried host gene fragment as to the viral genome, and also the endogenous host gene target.

numerous viruses, including potyviruses, cucumoviruses, and tobamoviruses, are able to counteract these mechanisms by inhibiting this type of PTGS. Therefore, the abundant expression of the dsRNA to trigger efficient RNA silencing becomes crucial for effective resistance. To achieve resistance, inverse repeat sequences from viral genomes were widely used to form hairpin dsRNA *in vivo*, including small hairpin RNA (shRNA), self-complementary hpRNA, and intron-spliced hpRNA. Among these methods, self-complementary hairpin RNAs separated by an intron likely elicit PTGS with the highest efficiency. The presence of inverted repeats of dsRNA-induced PTGS (IR-PTGS) in plants also showed high resistance against viruses. IR-PTGS is not required for the formation of dsRNA for the processing of primary siRNAs, but the plant RNA-dependent RNA polymerases (RDRs) are responsible for the generation of secondary siRNAs derived from non-transgene viral genome, which further intensify the efficacy of RNA silencing induced by hpRNA, a process named RNA silencing transitivity. Among them, the sequence similarity between the transgene sequence and the challenging virus sequence is the most important. Scientists have engineered several transgenic plants with multiple hpRNA constructs from different viral sources, or with a single hpRNA construct combining different viral sequence. Thus, multiple viruses can be simultaneously targeted, and the resulting transgenic plants show a broader resistance with high efficacy. In addition to the sequence similarity, the length of the transgene sequence also contributes to high resistance. In general, an average length of 100–800 nt of transgene sequence confers effective resistance [37]. Various transgenic crop plants have been developed for enhanced resistance to number of viruses using variety of strategies (Table 1).

Engineered crops resistance to herbicide tolerance: Engineering herbicide tolerance in transgenic plants has been accomplished exploiting at least three different mechanisms: (I) overexpression of the target enzyme, (II) modification of the target enzyme, and (III) herbicide detoxification [38]. Examples of transgenic plants developed based

Table 4: Recent developments of transgenic crop plants with improved nutritional quality, oil production etc.

Sl. No.	Transgene	Function	Source of Transgene	Crop and Cultivar
1.	dgat1-1	acetyl glyceride oil production	*Arabidopsis thaliana*	*Camelina sativa*
2.	gus and nptII	High seed production	*E. coli*	*Camellia sinensis* L.O. Kuntze
3.	FAD2	Oleic acid production	Linum usitatissimum L. cDNA library	Linum usitatissimum L.
4.	OtΔ6 (Δ6-desaturase gene), PSE1, (TcΔ5) Δ5-desaturase gene, PsΔ12 (Δ12-desaturase gene), Pi-x3(x3-desaturase)	omega-3 LC-PUFA production	Ostrococcustauri, Physcomitrella patens, Thraustochytrium sp., Phytophthorasojae, Phytophthorainfestans	Camelina sativa

on each mechanism are following. Glyphosate is an environmentally more benign, widely used broad-spectrum herbicide. It is easily degraded in the agricultural environment and works by interfering with the EPSPS enzyme system that is present only in plants. Unfortunately, the herbicide kills crop plants as well as weeds. Transgenic plants including maize, soybean, and cotton have been developed, overexpressing an additional copy of the EPSPS gene from *Petunia hybrid* under the strong 35S promoter and exhibiting increased tolerance to glyphosate. Alternatively, expression of a mutant *Aro A* gene from *Salmonella typhimurium* (which encodes EPSPS) in transgenic tobacco resulted in even higher tolerance to the herbicide than overexpression of the wild-type petunia EPSPS gene [38]. This would allow farmers to control weeds in transgenic cultivars spraying with glyphosate alone. Recently, another approach has been employed for the development of resistance to the herbicide phosphinothricin (basta). The *bar* gene from *Streptomyces hygroscopicus* or *S. uiridochromogenes* encodes the enzyme phosphinothricin acetyl transferase (PAT), which converts the herbicide to a nontoxic acetylated form. Expression of the *bar* gene in transgenic tobacco, potato, and tomato plants conferred phosphinothricin resistance at up to 10 times the normal application rate of the hebicide in the field [39]. Transgenic technologies have been used extensively to modify other important characteristics of plants such as starch composition in potato, ripening in tomato, lignin content in arabidopsis, flower vase-life in carnation and explore many new possibilities for their uses in agriculture as well as in industry [40].

Engineered crops resistance to abiotic stresses: Plant responses to different stresses are highly complex and involve changes at the transcriptome, cellular, and physiological levels. Recent evidence shows that plants respond to multiple stresses differently from how they do to individual stresses, activating a specific programme of gene expression relating to the exact environmental conditions encountered [41]. Rather than being additive, the presence of an abiotic stress can have the effect of reducing or enhancing susceptibility to a biotic pest or pathogen, and vice versa. This interaction between biotic and abiotic stresses is orchestrated by hormone signalling pathways that may induce or antagonize one another, in particular that of abscisic acid. Specificity in multiple stress responses is further controlled by a range of molecular mechanisms that act together in a complex regulatory network [41-42]. Transcription factors, kinase cascades, and reactive oxygen species are key components of this cross-talk, as are heat shock factors and small RNAs. Identifying master regulators that connect both biotic and abiotic stress response pathways is fundamental in providing opportunities for developing broad-spectrum stress-tolerant crop plants [42]. Table 3 represents the attempts made in the developments for the abiotic stress tolerant crops in details.

Nutritional enhanced GM feed crops: Genetic modification especially on the purposeful changing of substances in a particular pathway using recombinant DNA techniques, termed as metabolic engineering, is being conducted to generate new varieties with high yielding and nutrition-enhanced traits. Nutrition enhancement in crops targets manipulation of levels of proteins and amino acids, fats and oils, vitamins and minerals, carbohydrates and fiber quality, as well as decreasing the levels of undesirable components in major feed crops (Fig. 4) [43]. GM maize with increased lysine (LY038) was developed by inserting a *cordapA* gene from a

common soil bacteria *Corynobacterium glutamicum*. Enhanced production and accumulation of free lysine (Lys) in the GM corn kernel made body weight gain, feed conversion and carcass yields of experimental poultry and swine comparable with animals fed with Lys supplemented diets, and higher than those fed with conventional maize diets. Barley with its inherent high ß-glucan content has not been used as a feed component. However, with the expression of a thermo-tolerant *Bacillus* ß-glucanase that acts on these glucans, GM barley could be a possible alternative or addition to feeds especially in areas where maize cannot be grown for climatic reasons [44]. Most of the GM crops modified to improve fatty acid content have been used for direct food or for food industry use such as the oleic acid soybean DP305423, which has a better oxidative ability for improved food frying performance [43]. The preceding overview of nutritionally enhanced feed crops developed through genetic modification provided information on crops and traits that are under field trial or are already in the early commercialization stages. Nutritionally-enhanced genetically modified feeds have consistently shown efficacy in providing safe and available nutrients to poultry and livestock in various studies. Sufficient and cheap feedstock is expected to come as more countries are adopting biotech crops. Research on increasing other nutrients in feed crops such as vitamins, minerals, and fats, reducing anti nutrition factors in plant-based feeds, efficient anaerobic fermentation of silage through genetically modified microorganisms will surely contribute to this endeavour. The preceding overview of nutritionally enhanced feed crops developed through genetic modification provided information on crops and traits that are under field trial or are already in the early commercialization stages [43,44].

CONCLUSION

World's "ballooning" population including global climate change, limited availability of arable land, and various biotic and abiotic stress factors threatening crop production worldwide. Conventional breeding alone may not be sufficient to meet the forth coming demand. The current scenario of 'gene revolution' coupled with modern tools of plant biotechnology is in now a state to effectively complement conventional plant breeding in an economically useful way to genetically improve crop plants. In the past decade, an array of transgenic crop plants has been developed in different laboratories across the world for enhancement of resistance to biotic stresses, viral disease resistance and biofortification of produce, herbicide tolerance and increase yield in crop plants. The awareness programme should be undertaken to educate producers, consumers and government regulatory agencies regarding benefits offered by transgenic crops and possible ricks to increase the basic research as well as area under transgenic crop cultivation.

REFERENCES

[1] Conner, A.J., Glare. T.R. and Nap, J.P.: Plant J. 33(1):19-46. (2003).

[2] Meagher, R.B.: Current Opinion in Plant Biology. 3(2):153–162. (2000).

[3] Roush, R.T.: Pestic. Sci. 51 (3): 328–34. (1997).

[4] Herrera-Estrella, L., Depicker, A., Montagu, M.V. and Schell, J.: Nature. 303, 209 – 213. (1983).

[5] Grunewald, W., Bury, J. and Inzé, D.: Nature. 497,40. (2013).

[6] Park, S.H., Pinson, S.R.M. and Smith, R.H.: Plant Mol. Biol. 32:1135-1148. (1996).

[7] Tester, M., and Langridge, P.: Science. 327(5967):818-22. (2010).

[8] Uzogara, S.G.: Biotechnology Advances. 18. 179–206. (2000).

[9] Alfonso-Rubi, J., Ortego, F.P., Carbonero, P. and Diaz, I.: Transgenic Res., 12: 23-31 (2003).

[10] Abel, P.P., Nelson, R.S., De, B., Hoffman, N. and Rogers, S.G.: Science. 232: 738-743 (1986).

[11] Bano-Maqbool, S., Riazuddin, S., Thi-Loc, N., Gatehouse, A.M.R., Gatehouse, J.A., and Christou, P.: Mol. Breed. 7:85-93. (2001).

[12] Constantin, G.D., Krat, B.N., MacFarlane, S.A., Nicolaisen, M., Johansen, I.E., and Lund, O.S.: The Plant Journal. 40(2):622–631. (2004).

[13] Dalrymple, M.A. Biotechnol and Genet Eng Rev. 15:33–49. (1998).

[14] Fraley, R.T., Rogers, S.G., Horsch, R.B., Sanders, P.R., Flick, J.S., Adams, S.P., Bittner, M.L., Brand, L.A., Fink, C.L., Fry, J.S., Galluppi, G.R., Goldberg, S.B., Hoffmann, N.L. and Woo, S.C.: Proc. Nat. Acad. Sci. USA. 80(15): 4803–4807. (1983).

[15] Vaeck, M., Reynaerts, A., Hofte, H., Jansens, S., Beukeleer, M.D., Dean, C., Zabeaug, M., Montagu, M.V. and Leemans, J.: Nature. 328(1): 33–37. (1987).

[16] Bruening, G. and Lyons, J.M.: California Agriculture. 54(4):6–7. (2000).

[17] Manoj, B., Zhu, L., Shen, G., Payton, P. and Zhang, H.: Plant Biotechnol Rep. 6:59–67. (2012).

[18] Edwin, A.G., Vossen, V., Gros, J., Sikkema, A., Muskens, M., Wouters, D., Wolters, P., Pereira, A. and Allefs, S.: The Plant Journal. 44,208–222. (2005).
[19] Sudarshana, M.R., Roy, G. and Falk, B.W.: Methods Mol. Biol. 354:183-196. (2006).
[20] Bates, S.L., Zhao, J.Z., Roush, R.T. and Shelton, A.M.: Nat. Biotechnol. 23:57-62. (2005).
[21] De Cleene, M. and De Ley, J.: The host range of crown gall. Bot. Rev. 42:389-466. (1976).
[22] Walden, R. and Schell, J.: Eur. J. Biochem. 192: 563-576. (1990).
[23] Maagd, R.A., Bosch, D. and Stiekema, W.: Trends Plant Sci. 4 (1), 9–13. (1999).
[24] Kuc, J.: Phytoparasitica. 18 (1), 3–7. (1990).
[25] Tohidfar, M. and Khosravi, S.: Biotechnol. Agron. Soc. Environ. 19(1), 62-70. (2015).
[26] Ahmed, S.A., Dutta, L.C. and Sarmah, M.C.: Acad. J. Entomol. 5(2): 94-98 (2012).
[27] Yamamoto, T., Iketani, H., Ieki, H., Nishizawa, Y., Notsuka, K., Hibi, T., Hayashi, T. and Matsuta, N.: Plant Cell Reports. 19(7):639-646. (2000).
[28] Takakura, Y., Ito, T., Saito, H., Inoue, T., Komari, T. and Kuwata, S.: Plant Molecular Biology. 42,(6): 883-897. (2000).
[29] Rohini, V.K. and Rao, K.: Plant Science**.** 160, (5):889–898. (2001).
[30] Baulcombe, D. RNA Silencing in Plants. Nature. 431 (2), 356–363. (2004).
[31] Turnage, M.A., Muangsan, N., Peele, C.G. and Robertson, D.: Plant J. 30 (1), 107–114. (2002).
[32] Voinnet, O. Trends Genet. 17 (8), 449–459. (2001).
[33] Peter, M. and Helliwell, W.C.: Nature Rev. Genet. 4 (1), 29–38. (2014).
[34] Kjemtrup, S., Sampson, K.S., Peele, C.G., Nguyen, L.V., Conkling, M.V., Thompson, W.F. and Robertson, D.: Plant J. 14 (1), 91–100. (1988).
[35] Peele, C., Jordan, C.V., Muangsan, N., Turnage, M., Egelkrout, E., Eagle, P., Hanley-Bowdoin, L. and Robertson, D.: Plant J. 27 (4), 357–366. (2001).
[36] Ding, S.W.: Nat. Rev. Immunol. 10 (9), 632–644. (2010).
[37] Himber, C., Dunoyer, P., Moissiard, G., Ritzenthaler, C. and Voinnet, O.: EMBO J. 22 (1), 4523–4533. (2003).
[38] Tsaftaris A. Field Crops Res. 45: 115-123. (1996).
[39] Wohlleben, W., Arnold, W., Broer, I., Hillemann, D., Strauch, E. and Puhler, A.: Gene. 70: 25-37. (1988).
[40] Ni, W.T., Paiva, N.L. and Dixon, R.A.: Transgenic Res. 3:120-126. (1994).
[41] Atkinson, N.J. and Urwin, P.E. : J Exp Bot. 1: 21. (2012).
[42] Rejeb, I.B., Pastor, V. and Mauch-Mani, B.: Plants. 3:458-475. (2014).
[43] Chen, G., Zhang, B. and Zhao, J.: Materials. 8(1):2346-2360. (2015).
[44] Franco, O.L., Rigden, D.J., Melo, F.R. and Grossi-de-Sá, M.F.: The FEBS journal. 269(2): 397–412. (2002).