

Transgenic plants: resistance to abiotic and biotic stresses

AKILA WIJERATHNA-YAPA

*Division of International Studies, Robert H. Smith Faculty of Agriculture, Food and Environment,
The Hebrew University of Jerusalem, Israel.*

Corresponding author: akila.yapa@mail.huji.ac.il

Submitted on 2017, 25 May, accepted on 2017, 5 June. Section: Reviews

Abstract: Today's crop breeding combined with improved agricultural management has brought substantial increases in food production. But irrigation, fertilizers pest management requires a high energy input that creates a drain on the already scarce fossil fuels. It is thus clear that different strategy has to be adopted to increase crop productivity further to meet the needs of rapidly increasing world population. Crop breeders are endeavoring to meet this challenge by developing crops with higher yield, better resistance to pest, disease and weedicides, tolerance to various stress conditions.

Key words: *Transgene action, herbicide resistance, insect resistance, virus resistance, disease resistance, drought resistance*

Introduction

Crop improvement through the conventional breeding approaches is hindered due to narrow genetic variability and natural barriers of crossing among existing species. Though, the mutational breeding and somaclonal variation technologies seek to increase the existing genetic variability, the success is unpredictable and random. However, the plant genetic engineering and tissue culture techniques have been highly recognized as the advanced and much effective breeding tools in crop improvement programs.

In genetic engineering, the selected useful individual genes from any living organism can be transferred into a desired crop plant and obtaining a proper expression. Hence, genetic engineering of plants is rapidly becoming a productive field while creating novel varieties with a new combination of genes and genetic engineering technologies are more effective genetic manipulation compared to the conventional breeding methods. . Conventional breeding methods have to apply with whole organism while the new breeding technologies operate at cellular and molecular level. Moreover, in genetic engineering, the gene transformation and protoplast fusion allow to bypass sexual reproduction and move desirable gene between completely unrelated organisms, while conventional breeding relies upon sexual reproduction

to transfer genetic materials. Genetic engineering always permits modification of living organisms with an unprecedented specificity and allow a qualitatively different degree of genetic transformation.

Although there are enormous advantages have been encountered with genetic engineering, several limitations have also been recognized. The lack of efficient transformation and regeneration systems, especially for monocots, which include world's major cereal crops, is one of the limitations in plant genetic engineering. Further, the paucity of agronomically important and useful genes which when transferred with appropriate molecular controls would confer beneficial traits on recipient crop plant is recognized as another limitation to the commercial development of genetic engineering of plants. In addition, the success of genetic engineering in monocots and legumes is hindered due to the inability to regenerate whole plants from transformable cells.

Gene manipulation techniques coupled with conventional breeding programs are expected to result in great improvements in crop production. Successful first steps towards the introduction of disease, herbicide and pesticide resistance in plants have already been reported from laboratories using genetic engineering and tissue culture methodologies.

Transgene action

The limitations identified in selective breeding can be overcome through the gene manipulation with transgenic technology and it allows to increase the genetic diversity as well. The *in vitro* genetic manipulation techniques of plant cells and tissues were being developed in late 1970 and onwards. The directed desirable gene, transfer across taxonomic boundaries and subsequent expression of the gene is referred as *transgenesis*. The transferred gene is known as *transgene* and organism that resulted after successful gene transferring is known as *transgenic organism*. The gene transfer techniques in plants have been developed very fast and today, techniques are available which rely upon plant vectors as well as vector-less systems which includes directed physical and chemical methods for introducing foreign DNA into plant cells.

Herbicide resistance

In modern agriculture, the herbicides have been taken the major role in weed control. Though the uses of herbicide offers several advantages, i.e., permitting economic weed control, increasing the efficiency of crop production resulting in higher crop yield and biodegradability etc., they are endowed with several limitations as well, i.e., lack of selectivity is one of the most important factor. Most of the herbicides distinguish between weeds and crops, and non-selectivity limits their use to a greater extent.

Genetic engineering offers the scope of modifying plants through integration of genes providing resistance to broad spectrum herbicides. As consequences, a major effort has been devoted in several laboratories to create herbicide resistant plants as it is governed by single genes. Three approaches have been followed in the production of herbicide resistant plants: i) over production of herbicide sensitive biochemical targets; ii) structural alteration of a biochemical target resulting in reduced herbicide affinity, and iii) detoxification degradation of the herbicide before it reaches the biochemical target inside the plant cell.

Herbicide resistant plants can develop by introducing genes that produce an enzyme which degrade the herbicide sprayed on the plants. Introduction of *bar* gene cloned from bacteria *Streptomyces hygroscopicus* into plants, make them resistant to herbicides based on phosphinothricin (Padgette *et al.*, 1995). According to Padgette *et al.* (1995), the *bar* gene produces an enzyme, i.e., *phosphinothricin acetyl transferase (PAT)* which degrades phosphinothricin into a non-toxic acetylated form (The gene *bxn* has identified in *Klebsiella ozaenae* which produces *nitrilase* enzyme which imparts the resistance to plants against herbicide Bromoxynil (Tan *et al.*, 2006). Other genes including *tfdA* for 2, 4-D tolerance (Bayley *et al.*, 1992) and Glutathione S-transferases (GST) gene for Atrazine tolerance have also been discovered (Jepson *et al.*, 1997).

Another way of developing herbicide resistant plants is the transferring of gene responsible for an insensitive enzyme to herbicide (target modification). In this approach, a mutated gene is introduced which produces modified enzyme in the plant which is not recognized by the herbicide; hence the herbicide cannot kill the plant. A mutant *aroA* gene from bacteria *Salmonella typhimurium* has been used for developing tolerance to herbicide; glyphosate (Fillatti *et al.*, 1988). Further, a tolerance to herbicides has been achieved by engineering the expression of the mutant herbicide Acetolactate synthase (*ALS*) gene derived from plant (Chipman *et al.* 1998).

Table 1 - Broad-spectrum herbicides and the resistant genes.

HERBICIDE RESISTANCE	GENE	SOURCE
Glufosinate, Phosphinothricin bialaphos	<i>bar</i> , PAT (phosphinothricin acetyl transferase)	<i>Streptomyces sp.</i> <i>Alcaligenes sp.</i>
Glyphosate	<i>aroA</i> , EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene	<i>Salmonella typhimurium</i> <i>Agrobacterium sp. strain CP4</i>
Bromoxynil	BXN (Bromoxynil nitrilase)	<i>Klebsiella pneumoniae</i>
Sulfonylurea	ALS (acetolactate synthase)	<i>Nicotiana tabacum</i>
2, 4-dichlorophenoxy acetate (2,4-D)	<i>tfdA</i> (2,4-D monooxygenase)	<i>Ralstonia eutropha</i>

Glyphosate Action

Glyphosate (N-(phosphonomethyl) glycine), is used to control the wide range of weeds and is a phosphomethyl derivative of the amino acid glycine. This inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which is present in fungi and bacteria, but not in animals. EPSPS catalyzes a key step in the synthesis of aromatic amino acid hormones and other critical plant metabolites by transferring the enolpyruvyl moiety of phosphoenol-pyruvate to shikimate-3-phosphate. The active site of the EPSPS enzyme in higher plants is very highly conserved. More interestingly, the binding site for glyphosate is closely overlapped with the binding site of phospho-enolpyruvate while having a unique mechanism of inhibition (Dill *et al.*, 2010).

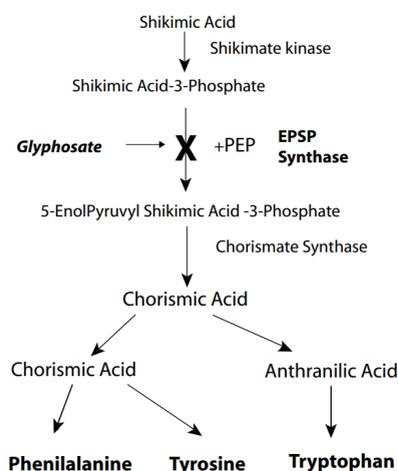


Figure 1- Glyphosate mode of action (Adapted from Dill *et al.*, 2005)

The control of underground corms, rhizomes and other potential vegetative structures of weeds can be resulted due to the translocation ability of glyphosate in growing meristematic tissue and inhibit an enzymatic process present in plants. Glyphosate was initially used to control perennial weeds on ditch bank in right of way and follow fields owing to its unique properties. However, the utilization of glyphosate was limited to the main stream agriculture since, it kills the main crop as well and further, glyphosate is used for land preparation without tilling (Dill *et al.*, 2010).

Strategies for Glyphosate Resistance

Development of broad spectrum herbicide glyphosate resistant crops has greatly improved agricultural efficiency throughout the world. Tolerance to this herbicide is obtained either by i) over production of enzyme EPSPS or ii) degradation of

glyphosate into aminoethyl phosphonic acid, a non-toxic compound. Both types of mutations have been selected in *Salmonella typhimurium* (Comai *et al.*, 1983).

- i) *Over production of enzyme EPSPS*: a gene *aroA* from *Salmonella typhimurium* encoding EPSPS was isolated, cloned and sequenced (Stalker *et al.*, 1985) and transferred to tobacco (Comai *et al.*, 1983) and tomato (Fillatti *et al.*, 1987) which showed tolerance to glyphosate. *aroA* gene obtained from *E. coli* has also been used for generation of transgenic tobacco plants (Della-Cioppa *et al.* 1987). Another EPSPS gene derived from *Petunia hybrida* yielded glyphosate tolerant *Petunia* cell lines (Steinrucken *et al.*, 1986). Roundup ready plants carry the gene coding for a glyphosate-insensitive form of this enzyme, obtained from *Agrobacterium sp. strain CP4*. Once incorporated into the plant genome, the gene product, CP4 EPSP synthase, confers crop resistance to glyphosate.
- ii) *Detoxification of the glyphosate*: a glyphosate oxido-reductase (*gox*) gene was isolated from a bacterium and it degrades glyphosate in to non-toxic aminoethyl phosphate (Barry *et al.*, 1992). Transformants have been obtained using *CP4 EPSP* and *gox* in wheat through particle bombardment of embryos (Zhou *et al.* 1995).

Table 2 - Glyphosate resistant transgenic plants developed from various species

SPECIES	GENE	METHOD	MODE OF ACTION	REFERENCE
Petunia	EPSPS	At	Overproduction of EPSPS	Steinrucken <i>et al.</i> , 1986
Tobacco	<i>aroA</i>	At	Overproduction of EPSPS	Comai <i>et al.</i> , 1985
Tomato	<i>aroA</i>	At	Overproduction of EPSPS	Della-Cioppa <i>et al.</i> , 1987
Soybean	CP4-EPSPS	PB	Overproduction of EPSPS	Padgett <i>et al.</i> , 1995
Poplar	EPSPS	At	Overproduction of EPSPS	Filliati <i>et al.</i> , 1987
Wheat	CP4-EPSPS and <i>gox</i>	PB	Overproduction of EPSPS and detoxification	Zhou <i>et al.</i> , 1995

At - *Agrobacterium tumefaciens*; PB - Particle bombardment

Insect resistance

The transgenic technology provides an alternative and innovative method to improve pest control management which is eco-friendly, effective, sustainable and beneficial in terms of yield. The well-known insect resistant approach is the introduction of *Bacillus thuringiensis* bacterial gene *Bt* synthetic *Bt* and introduction of plant gene(s) for insecticidal proteins. The other genes which are used for insect control includes Cowpea trypsin inhibitor (CpTI), Alpha amylase inhibitor (AI), snowdrop lectin (*Galanthus nivalis* agglutinin: GNA), protease inhibitor II gene (Pin II) etc. A list of insect resistant plants has been shown in table below.

Table 3 - Several transgenic plants conferring resistance against insects

GENE TRANSFERRED	CROP	INSECTS CONTROLLED	REFERENCES
cry1H (Bt toxin)	Maize	European corn borer	Jansens, 1997
Barley trypsin inhibitor	Rice	Insect resistance	Alfonso-Rubi <i>et al.</i> , 2003
cryIIIB (Bt toxin)	Eggplant	Leptinotarsa decemlineata	Iannacone <i>et al.</i> , 1997
Cowpea serin PI	Rice	Stem borer	Duan <i>et al.</i> , 1996
Snow drop lectin	Potato	Potato aphid	Gatehouse, 1997
cry1A (Bt toxin)	Soybean	Insect resistance	Macrae <i>et al.</i> , 2005
cryIAC	Chickpea	Insect resistance	Sanyal <i>et al.</i> , 2005
cryIAb (Bt toxin)	Cotton	Cotton bollworm	Tohidfar <i>et al.</i> , 2008
cry3a (Bt toxin)	Alfalfa	Insect resistance	Tohidfar <i>et al.</i> , 2013

Introduction of bacterial gene Bt synthetic Bt

The entomopathogenic *Bacillus thuringiensis* (*Bt*) produces proteinaceous crystalline (Cry) inclusion bodies during sporulation and also produces cytotoxins that synergize the activity of Cry toxins. Cry proteins are toxic to insects (mainly against lepidopteran, coleopteran, dipteran, and nematodes), but non-toxic to human and animals (BANR, 2000). These toxins are thought to aggregate and form ion permeable pores that lead to gut dysfunction, lysis of gut epithelial cells, and the eventual death of the insect. The specificity of insecticidal activity of *Bt* on a particular insect species is determined by the form(s) of the cry gene(s) carried by the bacterium. *Bt* lepidopteron specific from *B. thuringiensis* sub sp. Kurstaki has been widely and successfully used in tobacco, tomato, potato, cotton, rice and maize for developing resistance against several lepidopteron insect pests (BANR, 2000).

Toxic Action of Cry Proteins

When ingested by lepidopteran insect larvae the Cry protein, a protoxin, is solubilized by the high pH of the gut lumen and solubilization of the protoxin is activated through cleavage by digestive enzymes into a smaller (~60kDa) fragment (Hofte and Whiteley, 1989; OECD, 2007). Then, activated toxic fragment can binds to receptors on the membrane of the insect's midgut epithelial cells (Bravo *et al.*, 1992) and follows the activation of an apoptotic signal cascade pathway (Zhang *et al.*, 2006), causing loss of homeostasis by formation of pores (Figure 2). This leads to osmotic shock, cell lysis, septicemia, and insect death (Lorence *et al.*, 1995). In some species enteric bacteria are required for insect death (Broderick *et al.*, 2006, 2009).

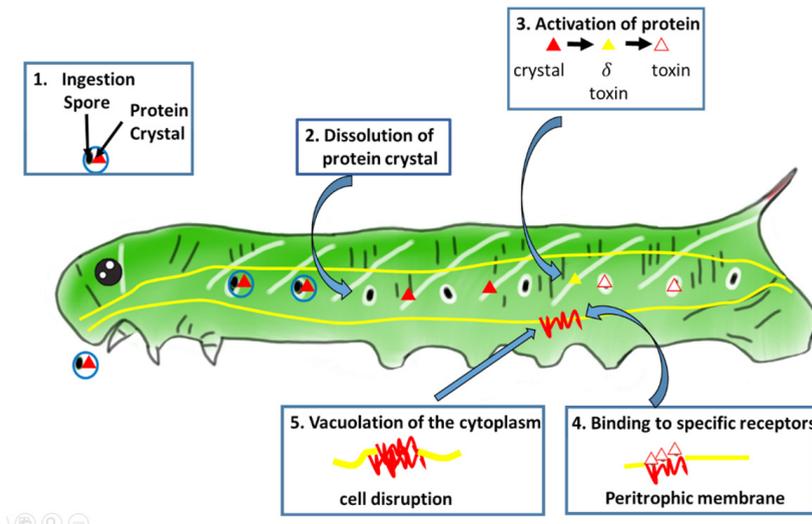


Figure 2 Mode of action of *Bacillus thuringiensis* in Lepidopteran caterpillar: 1. ingestion of bacteria; 2. solubilization of the crystals; 3. activation protein; 4. binding of proteins to the receptors; 5. membrane pore formation and cell disruption (Modified from: Schünemann *et al.*, 2014).

The Crystal (Cry) Proteins

The δ -endotoxins, a major source of the bacterium's toxicity are composed of one or more crystal (Cry) and/or cytolytic (Cyt) proteins and produced in the sporulation phase (Bravo *et al.*, 2007). The toxicity, mode of action, and specificity of Cry proteins have been experimentally verified (OECD, 2007). *CRY* genes constitute nearly all the anti-insect genes in transgenic insect protected plants. Different types of Cry proteins which belong to distinct protein families have been identified and these holoproteins are range in size from 50 to 140kDa (Crickmore *et al.*, 1998). Moreover, binary forms of Cry proteins occur in the bacterium are used in transgenic crops. The best characterized Cry34A/Cry35A binary protein has constituent masses of 14 and 44 kDa, respectively (Schnepf *et al.*, 2005). Most Cry proteins have a distinct specificity and target only a single order or a few species from that order. Some, however, have a broader spectrum of activity that spans two or three orders.

Based on their host range Hofte and Whiteley classified Bt toxins into 14 distinct groups and 4 classes (Hofte and Whiteley 1989) viz.

- CryI (active against Lepidoptera)
- CryII (Lepidoptera and Diptera)
- CryIII (Coleoptera) and
- CryIV (Diptera).

Cry proteins are organized into three main groups' based on the structure and function viz. the three-domain, the mosquitocidal-like, and the binary-like Cry toxins. Three-domain Cry proteins are the largest group and the majority of the Cry toxin genes used to transform plants to impart insect resistance belong to this group. The three-domain group is further divided into more than 40 different types with many different subgroups (Crickmore *et al.*, 1998). New three-domain Cry proteins are assigned to a group primarily based on their sequence. Domain I of the Cry protein is responsible for pore formation and the other two domains determine the insect specificity of the toxin.

CRY Genes Expression in Plants

The first generation of transgenic plants containing cry genes provided high levels of δ -endotoxin in all plant tissues. Through replacement of constitutive promoters, such as the CaMV 35S promoter, with wound-inducible (Vaeck *et al.*, 1987), chemically-inducible (Williams *et al.*, 1992) and tissue-specific promoters (Koziel *et al.*, 1993), the second generation Bt-crops will incorporate some aspects required to address resistance management (Whalon *et al.*, 1993a). Development of resistance to Bt toxins is one of the main concerns related to use of Bt-expressing transgenic plants. Laboratory selection for resistance to Bt δ -endotoxin has been demonstrated for lepidoptera (McGaughey 1985; Tabashnik *et al.*, 1991), coleoptera (Whalon *et al.*, 1993b), and diptera (Goldman *et al.*, 1986). However, to date the diamondback moth, *Plutella xylostella*, a pest of cruciferous plants, is the only insect reported to have developed high levels of resistance in the field (Tabashnik *et al.*, 1990; Ferre *et al.*, 1991). Transgenic plants expressing active toxins directly remove requirements for specific gut conditions required to activate the protoxin; this could potentially expand the range of non-target hosts (Addison *et al.*, 1993).

Bt maize has been transformed with either *cry1Ab*, *cry1Ac* or *cry9C* to protect it against *Ostrinia nubilalis* and *Sesamia nonagriodes*, or with *cry1F* to protect it against *Spodoptera frugiperda*, and with *cry3Bb*, *cry34Ab* and *cry35Ab* to protect it against the rootworms of the genus *Diabrotica* (James, 2012). Most commercially planted *Bt* cotton contain *cry1Ac* or a fusion gene of *cry1Ac* and *cry1Ab* (James, 2013). *Bt* potatoes protected against *Leptinotarsa decemlineata* have also been planted commercially in North America and Europe and contain the *cry3Aa* gene (Coombs *et al.*, 2002).

In 2000 India commercialize *Bt* eggplant. *Bt* crucifer vegetables are under development and are targeted against *Plutella xylostella*. Also, *Bt* alfalfa has been produced using *cry3a* gene against *Hypera postica* for the first time in Iran (Tohidfar *et al.*, 2013). Finally, the *Bt* trait has been introduced in soybean through either one or two cry genes among *cry1Ab*, *cry1Ac*, *cry1F* (James, 2013).

Based on the crystal protein gene sequence of *B. thuringiensis sub sp. Kurstaki* (*Btk*) strain HD-1, *cryIA(c)* synthetic gene which consists nearly identical amino

acids portions to the natural environment was produced and this corresponds to a protein in commercial *Btk* formulations (e.g. Dipel®). A gene promoter (35S) from the Cauliflower Mosaic Virus was added that turns the gene on and produces the RNA leading to the production of the *Bt* protein in the plant (more specifically in the ribosomes of the plant cells). A marker gene was added to the gene construct, the product of which enables the identification of tissue cultured cell lines with stably integrated foreign DNA. The *nptII* gene was used, conferring resistance to the aminoglycoside antibiotics (kanamycin, neomycin, and G-418) which are inactivated after phosphorylation by *NPTII*. *NPTII* is produced in minute amounts in plants that contain the marker (Fraley *et al.*, 1986).

Other Genes for Insect Resistance

With Bt toxins being successfully engineered into crops, efforts are directed towards discovery of non Bt toxin genes having insecticidal activity. Several genes of plant origin such as protease inhibitors, lectins, amylase inhibitors can retard insect growth and development.

Protease Inhibitors

Plant protease inhibitors (PI) are able to protect plants against insect attacks by interfering with the proteolytic activity of insects' digestive gut. Among the proteic PIs, serine and cysteine PIs are abundant in plant seeds and storage tissues (Reeck *et al.*, 1997) and may contribute to their natural defense system against insect predation. The digestion of proteins in midgut is inhibited by PIs and cause mortality of insects due to nutritional imbalance (Broadway *et al.*, 1986; Ryan *et al.*, 1990). Further, the proteolytic activation of enzymes is blocked by PIs and some of metabolic processes (like moulting) are interfered (Hilder *et al.*, 1987). Also, the growth and development, multiplication rate, and insect life span are affected by PIs (Gatehouse *et al.*, 1999; Annadana *et al.*, 2002). The first PI gene that was successfully transferred artificially to plant species resulting in enhanced insect resistance was isolated from cowpea and encoded the trypsin/trypsin inhibitor CpTI (Cowpea Trypsin Inhibitor) (Hilder *et al.*, 1987). CpTI and Bt cotton cultivars were commercially released in China in 2000 (Song *et al.*, 2001) and accounted for approximately 15% of the grown cotton in 2005 (He *et al.*, 2008). Oryzacystatin 1 (OC1) is a well-studied cysteine PI from rice seeds which has been successfully introduced into several different crops like rice (Duan *et al.*, 1996), wheat (Altpeter *et al.*, 1999), oilseed rape (Rahbe *et al.*, 2003) and eggplant (Ribeiro *et al.*, 2006). It protects these plant species against beetle attacks and, in some cases, from aphids (Sharma *et al.*, 2004). A Bt-corn called Bt-Xtra containing three genes including cry1Ac from *B. thuringiensis*, bar from *Streptomyces higroscopicus* and potato proteinase inhibitor (pinII) has been produced (Oksman-Kaldentey *et al.*, 2002).

Lectins

Carbohydrate-binding proteins, lectins have identified in many plant tissues and are abundant in the seeds and storage tissues of some plant species. Plant lectins are particularly effective against the sap sucking Hemiptera (Powell *et al.*, 1995). Many transgenic plants expressing lectins have been developed to analyze the insecticidal properties under natural conditions. The toxic effects of different lectins range from a severe delay in development to high mortality in insects have been demonstrated on several insect species (Vandenborre *et al.*, 2011). Therefore, enhancing their presence in some plant tissues may have an insect tolerant effect. Transgenic rice with *Galanthus nivalis* (snow drop) agglutinin (GNA) has shown resistance to brown plant hopper (BPH) (*Nilaparvata lugens*) (Li *et al.*, 2005). *Allium* leaf agglutinin (ASAL) possesses an insecticidal activity in different plants. The ASAL gene was transferred to rice and the transgenic plants showed resistance to hopper insect pests (Saha *et al.*, 2006).

Alpha-amylase inhibitors

α -Amylases (α -1,4-glucan-4-glucanohydrolases) are hydrolytic enzymes, which catalyze the hydrolysis of α -1,4-glycosidic bonds in polysaccharides. They are present in microorganisms, animals and plants (Strobl *et al.*, 1998). α -Amylases are the most important digestive enzymes of many insects which feed exclusively on seed products. Inhibition of α -amylase impairs the digestion in an organism and causes shortage of free sugar for energy. α -Amylase inhibitors (α -AIs) are found in many plants as a part of the defense system and abundant in cereals and legumes (Iulek *et al.*, 2000). α -AIs are attractive candidates for the control of seed weevils because they are highly dependent on starch as energy source. The bean (*Phaseolus vulgaris*) amylase inhibitor gene was expressed in seeds of transgenic garden pea (*Pisum sativum*) and other grain legumes, using a strong seed-specific promoter (Shade *et al.*, 1994). The resulting seeds were resistant to stored product pests such as larvae of bruchid beetles and field pests such as larvae of the pea weevil *Bruchus pisorum* (Morton *et al.*, 2000). The alpha-amylase inhibitor gene isolated from *Phaseolus vulgaris* was introduced to chickpea by Agrobacterium-mediated transformation system (Ignacimuthu *et al.*, 2006). Although, the transformation efficiency was low (0.3%), the transformed plants showed a significant resistance to bruchid weevil. Similarly, *Coffea arabica* plants genetically modified with an alpha-amylase inhibitor gene isolated from *Phaseolus vulgaris* produced seed extracts capable of inhibiting amylolytic enzyme activity up to 88% (Barbosa *et al.*, 2010).

Virus resistance

Virus diseases of cultivated plants cause substantial loss in food, forage and fiber crops throughout the world. No large scale methods exist for curing plants once they have become virus infected. Thus control of viral diseases is dependent upon methods to prevent or delay the establishment of infection. Breeding for resistance is generally one of the most economical and practical methods, since it requires no additional labor or expense to the grower. The development of molecular strategies for the control of virus diseases has been especially successful owing to small genomic size of plant viruses which make them particularly amenable to molecular techniques for cloning. There are a number of different strategies for using molecular technology to integrate new resistance factors in plant virus systems. Transgenic plants produced for resistance through genetic transformation have been categorized into pathogen derived resistance category. The concept of pathogen derived resistance is based upon the idea that during an interaction with the host, the pathogen brings with it essential components and functions that are required for completion of its life cycle. These essential elements might then be disrupted by the presence of corresponding pathogen gene that is dysfunctional, over expressed or appears during the wrong stage of the life cycle of the pathogen. Thus, the objective of this approach is to identify those viral genes or gene product that when present at an improper time or in the wrong amount. This will interfere with the normal functions of the infection process and prevent disease development.

Virus Coat Protein Mediated Cross Protection

The concept of cross protection is the ability of one virus to prevent or inhibit the effect of a second challenge virus. Transgenic tobacco expressing tobacco mosaic virus (TMV) coat protein showed resistance similar to that occurs in viral mediated cross protection (Powell-Abel *et al.*, 1986). Since then number of coat protein genes from different virus groups have been found to provide resistance when expressed in transgenic plants (Table 4). Coat proteins, mediated resistance in many systems are correlated with the inhibition of virus replication at the initial point of infection. The resistance takes the form of reduced numbers of infection sites on inoculated leaves, suggesting that an initial step in the virus life cycle has been disrupted. It has been demonstrated that TMV cross protection may result from the coat protein of the protecting virus preventing un-coating of the challenge virus RNA. Coat protein mediated resistance may also function at a systemic level. The retardation in systemic movement and virus accumulation may involve a similar or different mechanism than what is responsible for resistance at the initial point of infection. Thus, the mechanisms involved in which coat protein mediated resistance has been reported

is directed against pulse-sense RNA viruses with a single capsid protein. This approach has been used in several crops like tobacco, tomato, potato, rice, maize, melons, alfalfa, sugar beet etc.

Table 4 Viral coat proteins used in resistance Transgenics

CROP	VIRUS CONTROLLED	REFERENCES
Squash	Cucumber Mosaic Virus (CMV)	USDA, 2000
Papaya	Papaya Ring Spot Virus (PRSV)	USDA, 2000
Soybean	Soybean dwarf virus (SbDV)	Tougou <i>et al.</i> , 2006
Alfalfa	Alfalfa mosaic virus (AMV)	Gomase and Kale, 2015
Tobacco	Tobacco mosaic virus (TMV)	Powell-Abel <i>et al.</i> , 1986
Tomato	Tobacco mosaic virus (TMV)	Powell-Abel <i>et al.</i> , 1986
Soybean	Bean pod mottle virus (BPMV)	Di <i>et al.</i> , 1996

Plant Virus resistance genes

A number of disease resistance genes (*R*) have been reported in crop plants against to the viral infections (Table 5). They encode products which respond to viral signals (avirulence (*avr*) gene products) culminating in a number of resistance responses in the plant.

Flor (1971) defined by the classical gene-for-gene hypothesis, which states that for every incompatible host pathogen interaction, there exist matching *R* genes in the host and *avr* genes in the pathogen. Resistance reaction against pathogen results generally by direct interaction between the products of *R* and *avr* genes. This interaction, in many cases, results in a resistance reaction, known as hypersensitive reaction (HR), which can be defined as a specific response of a host towards a pathogen. HR results in localized cell death, appearing as necrotic lesions at the site of pathogen entry. HR results in the arrest of pathogen spread, thereby effectively restricting it to the dead cells.

Satellite RNA

In addition to the tripartite messenger sense, single-stranded RNA genome, some strains of Cucumber Mosaic Virus (CMV) harbor satellite RNAs (satRNAs). The presence of sat-RNA modulates the symptoms induced by the helper virus (HV) and often depresses HV accumulation in different host species. CMV satRNA depends on its helper virus (HV) CMV for replication, movement within the plant, encapsidation and transmission (Baulcombe *et al.*, 1986).

Table 5 - R genes against viruses and corresponding avr gene products (Dasgupta et al., 2003)

RESISTANCE GENE	SOURCE PLANT	AVR PRODUCT OF THE VIRUS	PATHOGEN
<i>L2</i>	<i>Capsicum</i> sp	Coat protein	Pepper mild mosaic virus
<i>N</i>	<i>N. tabacum</i> cultivar <i>Samsun</i>	Replicase	Tobacco mosaic virus
<i>HRT</i>	<i>Arabidopsis thaliana</i> ecotype Dijon	Coat protein	Turnip crinkle virus
<i>Rx, Nx, Nb</i>	<i>Solanum tuberosum</i> cultivar <i>Cara</i>	Coat protein	Potato virus X
<i>TuRB01</i>	<i>Brassica napus</i>	Cylindrical Inclusion protein	Turnip mosaic virus
<i>Tm2</i>	<i>Lycopersicon esculentum</i>	Movement protein	Tobacco mosaic virus

Post-transcriptional gene silencing

Post-transcriptional gene silencing (PTGS) in plants is an RNA-degradation mechanism that shows similarities to RNA interference (RNAi) in animals. This, also called RNA interference or RNAi and results in down-regulation of a gene at the RNA level. In this mechanism, the elicitor double-stranded RNA (ds RNA), commonly produced during viral infection and degraded to 21–25 nucleotides, with the help of a variety of factors; termed as small interfering RNA (siRNA) (Plastere *et al.*, 2000). A complex of cellular factors, namely RNA-dependent RNA polymerase (RdRp) (Mourrain *et al.*, 2000), RNA-helicase (Dalmay *et al.*, 2001), translation elongation factor (Zou *et al.*, 1998), RNase (Ketting 1999) along with the small 21–25 nt RNA (of the elicitor RNA) acting as the guide RNA (Hammond *et al.*, 2001), supposedly degrade RNA molecules bearing homology with the elicitor RNA. This degradation process, initiating from a concerned cell having the elicitor RNA, spreads later within the entire organism in a systemic fashion. This process is generally regarded to have evolved as a plant defense mechanism against invading viruses containing either RNA (Smyth *et al.*, 1999) or DNA (Kjemtrup *et al.*, 1998) genomes.

Defective Viral Genomes

Defective interfering (DI) DNA are truncated genomic components which interfere with the replication of the genomic components. Their expressions of delayed disease symptoms and recovery, coupled with increased resistance upon repeated inoculation have been observed in plants engineered with DI DNA (Kunik *et al.*, 1994). For example, incorporation of sub-genomic DNA B that interferes with the replication of full length genomic DNA A and B confers resistance to ACMV in *N. benthamiana* (Frischmuth *et al.*, 1993).

Antisense RNA Approach

The replication strategy using the antisense approach attempts to block the replication of a virus by hybridization of complementary sequences to the replicase viral gene or to sequences recognized by the replicase during replication. Intransient assays with protoplasts of wheat, the antisense sequence of the first 250 nucleotides of the replicase gene of the geminivirus wheat dwarf mosaic virus (WDMV) completely inhibited virus replication (Gronenberg 1990). A second example involved the geminivirus tomato golden mosaicvirus (TGMV). The tobacco genome was integrated with the complete antisense sequence of the replicase gene of the TGMV and several lines were reported to exhibit a level of resistance when challenged with varying concentrations of TGMV (Lichtenstein and Buck 1990). Another example is that turnip yellow mosaic virus (TYMV), where antisense sequences corresponding to the tRNA-like structure of the 3' extremity of the TYMV RNA have been shown to strongly inhibit replicase activity (Cellier *et al.*, 1990). Transgenic plants that produce such sequences are under evaluation. However, application of this interesting approach must be further tested before it can be considered as a useful and practical strategy.

Ribozyme-Mediated Protection

A new approach to achieving virus resistance is the use of autocatalytic RNA cleaving molecules, known as 'ribozymes' (Cech 1986; Kim and Cech 1987). There is the possibility of self-cleavage during replication in viroid RNAs, (e.g. avocado sun blotch viroid; ASBV), and satellite RNAs (e.g. tobacco ring spot virus; TobRSV), (Buzayan *et al.*, 1986; Hutchins *et al.*, 1986; Prody *et al.*, 1986; Forster and Symons 1987). The sites of cleavage are intra molecular and presumably occur when the RNA molecule is in the correct configuration, thereby activating the cleavage reactions. Specific and effective cleavage on the positive and negative strand of the RNA is associated with conserved sequence domains. Several studies have been conducted to determine the optimal *in vitro* conditions of cleavage (Haseloff and Gerlach 1988; Gerlach 1989). Genes encoding sequences bearing specific virus cleavage sites have been integrated into transgenic plants and should generate sequence specific endonuclease activities. Constructs have been made to inactivate various viruses, including TMV and barley yellow dwarf virus (BYDV) (Gerlach 1989).

Resistance to fungal and bacterial diseases

Plant molecular biology and biotechnology techniques have taken a rapid progress in identification and cloning of genes involved in plant defense responses.

Further, genes and gene products that are involved in signaling pathways have also been predicted. So far, number of antifungal proteins and peptides have been isolated and assessed through *in vitro* bioassays. Transgenic plants have produced through different strategies viz. enhancement of plant structural defense, neutralization of fungal toxins and exploitation of antifungal genes from non-plant sources. Significant reductions of fungal diseases in many cases have been observed by exploitation of these approaches. Moreover, using the knowledge gathered from characteristics of these transgenic plants, it has been possible to obtain better resistance. Co-expression of multiple genes rather than single, use of inducible promoters instead of constitutive ones have been shown to give superior performance of transgenic plants. Further improvement in above strategies are however still necessary because all the above approaches have only resulted in varying degree of resistance, not complete fungus tolerance.

Ordinarily, hosts and pathogens show a “gene-for-gene relationship”. Dominant or semi-dominant resistance (*R*) gene in the plant, and a corresponding avirulence (*Avr*) gene in the pathogen are required for the incompatible interaction between plant and the pathogen. Discovery of the structure of *R* genes and *R* gene loci provides insight into *R* gene function and evolution, and should lead to novel strategies for disease control (Kim *et al.*, 1997). The gene-for-gene relationship classified into the following two general groups: (1) incompatible reaction and (2) compatible reaction.

Phyto-pathogenic bacteria generally have limited host ranges, often confined to members of a single plant species or genus. This appears to result from negative factors restricting the host range rather than from positive factors which allow the pathogen to infect its hosts. These negative factors are *Avr* genes present in the pathogen, which interact with matching resistance genes in the host. A cascade of responses is triggered in a plant when a pathogen carrying an *Avr* gene attacks the plant with the corresponding resistance gene and this results in localized host-cell death, preventing spread of the pathogen and the onset of disease (Klement, 1982). In the absence of either one or both of the matching gene pair, the plant fails to recognize the bacterium as a pathogen, the hypersensitive reaction (HR) is not triggered and disease will usually follow (Vivian *et al.*, 1997).

Gene-for-gene interactions determining race-cultivar specificity are proposed to be superimposed upon basic compatibility (Ellingboe, 1982). Following the studies on the interaction between flax and the rust fungus (*Melampsora lini*) Flor (1971) proposed that interaction of dominant, matching *Avr* and *R* genes in the pathogen and host, respectively. Ellingboe (1976) proposed that specificity lay in the direct interaction of gene products with the aid of the quadratic check, since if more than one gene were involved, the simple pattern of interaction would not obtain (Figure 3). The *Avr* gene product was envisaged to be the elicitor of the HR, interacting with a host receptor encoded by the resistance gene. Cloning and mutation of a number

of *Avr* and *R* genes have provided little support for such a model, since no *Avr* gene product has ever been shown to be secreted from the interior of the bacterial cell (Collmer, 1996). However in practice, the gene-for-gene hypothesis still provides a reliable basis to account for the outcome of plant-pathogen interactions.

$R \times avr^+$	 Incompatible system Resistant plant, Avirulent pathogen HR=Hypersensitive response	 HR
$R \times avr^-$ $r \times avr^+$ $r \times avr^-$	 Compatible system Susceptible plant, Virulent pathogen SR=Susceptible reaction	 SR

Figure 3 The incompatibility (occurring in many bio-tropic pathogenesis) and compatibility (found in many heterotrophic pathogenesis) types of gene-for-gene relationship in host-pathogen interactions. One gene for resistance (dominant) and one gene for virulence (recessive) are assumed.

Incompatible Reaction

Incompatibility (i.e. reduced pathogen development and reproduction associated with an effective host defense response) is expressed depends on the particular compatible gene pair viz., the plant receptor interacts with the pathogen molecule (Crute, *et al.* 1996). In order such interaction could occur, the plant and bacteria of a certain genotype should meet, i.e., a bacteria carrying the *avr*-gene interacts with a plant, which has the corresponding *R*-gene (Goryachkovsky *et al.*, 2000). Incompatible combination leads to quick progressing of events, or to hypersensitive response, further effects in the activation of plant defense responses, including localized host cell death, the hypersensitive response. The incompatible reaction is observed in case of obligate parasites, which associated with HR of the host and is triggered by certain unique molecules, called elicitors, of pathogen origin (Halterman *et al.*, 1997).

Compatible reaction

In this system, compatibility (i.e., extensive pathogen development and reproduction in the absence of an effective host defense response) is the outcome of a host-pathogen combination unless an allele for resistance at a particular host locus is specifically matched by an allele for a virulence at a particular pathogen locus (Crute *et al.*, 1996). In compatible reaction pathogen molecules are non-specific elicitors,

which are non-specific substances causing pathogenesis. Various external stimuli (wound, non-specific elicitors) activate protein kinases and genes of signal molecules biosynthesis (Goryachkovsky *et al.*, 2000). In the course of signal transduction, the synthesis of salicylic acid (SA), hydrogen peroxide (H_2O_2), jasmonic acid (JA), nitric oxide (NO), and ethylene (C_2H_2) is produced (Dixon R.A. *et al.*, 1995; Mauch-Mani B. *et al.*, 1996). In a compatible interaction (disease) the pathogen modulates pathogenicity targets in the host and manipulates gene regulation and signal transduction events to defeat the host defenses and locally modify the apoplast for bacterial colonization through nutrient release, water soaking and alkalization (Senthikumar *et al.*, 2013).

Strategies for Resistance

The key components of defense and offence mechanisms of many groups of fungi and bacteria are the antifungal and antibacterial proteins which are often effective on a broad range of targets and function synergistically in combinations, also with other biologically active compounds (Lorito *et al.*, 1996).

Overall transgenic approaches can be grouped into seven categories (Punja 2001, Grover *et al.*, 2003).

1. Over-expression of genes related to pathogenesis-related proteins and phytoalexins, which are directly toxic to pathogens or reduce their growth.
2. Expression of genes that destroy or neutralize the components of pathogen arsenal (e.g. polygalacturonase, oxalic acid and lipases).
3. Expression of gene products that enhance structural defense in the plants (e.g. peroxidase and lignin).
4. Expression of genes that regulate signals to control plant defenses (e.g. elicitor, SA, H_2O_2 , JA, NO and C_2H_4).
5. Expression of the resistance gene (*R*) products involved in HR for their interaction with *Avr* gene.
6. Expression of R gene that stopping invasion of fungus.
7. Application of RNAi technology RNAi, RNase and lysozyme.

Table 7 summarizes the selected list of work done on pathogenesis related proteins in transgenic plants.

Table 7 - Transgenic plants generated in various crops for resistance to disease

CROP	GENE TRANSFERRED	CONTROLLED PATHOGEN	REFERENCE
Tobacco	Bacterial chitinase gene from <i>Serratia marcescens</i>	<i>Rhizoctonia solani</i>	Jach <i>et al.</i> , 1992
		<i>Alternaria longipes</i>	Suslow <i>et al.</i> , 1988
	Bean chitinase gene	<i>Rhizoctonia solani</i>	Brogliè <i>et al.</i> , 1991
	Barley ribosome inactivating protein gene	<i>Rhizoctonia solani</i>	Jach <i>et al.</i> , 1992
	Barley α thionin gene	<i>Pseudomonas syringae</i> pv <i>tabaci</i>	Anzai <i>et al.</i> , 1989
Brassica napus	Bean chitinase gene	<i>Rhizoctonia solani</i>	Brogliè <i>et al.</i> , 1991
Potato	Bacteriophage T4 lysozyme	<i>Erwinia carotovora</i> subsp. <i>atroseptica</i>	During <i>et al.</i> , 1993
	H ₂ O ₂ gene for glucose oxidase	<i>Verticillium dahlia</i> <i>Phytophthora</i> spp. <i>Erwinia carotovora</i>	Wu <i>et al.</i> , 1995
Cucumber	Rice chitinase genes	<i>Botrytis cinerea</i>	Tabei <i>et al.</i> , 1998
Tobacco	Barley ribosome-inactivating protein	<i>Rhizoctonia solani</i>	Logemann <i>et al.</i> , 1992
Tobacco	Barley (<i>Hordeum vulgare</i>), a class II chitinase (CHI), a class II β -1,3-glucanase (GLU), and a Type-I ribosome inactivating protein (RIP)	<i>Rhizoctonia solani</i>	Jach <i>et al.</i> , 1995
Potato	osmotin gene	<i>Phytophthora infestans</i>	Liu <i>et al.</i> , 1994
Tobacco	PR-1 gene	<i>Perenospora tabacina</i> , <i>Phytophthora parasitica</i> var. <i>nicotianae</i>	Alexander <i>et al.</i> , 1993
Rice	Rice class I chitinase gene	<i>Rhizoctonia solani</i>	Ou <i>et al.</i> , 1985

Drought resistance

Wide array of physiological responses in plants are triggered by the drought, and affects the activity (either induced or repressed) of a large number of genes (Sahi *et al.*, 2006). Plants are sessile and exposed to the environmental changes and have to respond to their changing environment in a complex, integrated way at a given time.

Hence, responding to diverse environmental challenges are through the regulation of gene expression control is very complex and depend on the developmental stage of the plant (Sahi *et al.*, 2006).

Perception of drought stress and in the transmission of the stress signal accompanied by the activation of group of genes that encode proteins that protect the cells from the effects of desiccation (Shinozaki and Yamaguchi-Shinozaki 2007). Further, genes that govern energy-requiring water transport systems, passive transport across membranes, accumulation of compatible solutes, and protection and stabilization of cell structures are activated (Shinozaki and Yamaguchi-Shinozaki 2007). Another group of genes activated by drought is comprised by regulatory proteins that further regulate the transduction of the stress signal and modulate gene expression forming a highly complex and redundant gene network (Umezawa 2006; Shinozaki and Yamaguchi-Shinozaki 2007). Four independent stress-responsive genetic regulatory pathways have identified and two of the pathways are dependent on the hormone abscisic acid (ABA), where other two are ABA-independent. These pathways are also implicated in the perception and response to additional stress factors, including salinity and temperature variations.

Levels of the ABA in the plant greatly increase during the water stress and causing the stomatal closure. Thus, reducing the leaves water transpiration and activate stress response genes. The changing level of ABA in the plant reaction is reversible: once water becomes available again, the level of ABA drops, and stomata re-opens. Therefore, increasing the plant's sensitivity to ABA has been a very important target for improving drought tolerance.

Drought increases ABA levels and plant response to ABA is a crucial adaptive mechanism to overcome the drought stress (Robertson *et al.*, 1985; Uno *et al.*, 2000). Studies revealed that 9-cisepoxycarotenoid dioxygenase (NCED) is the critical enzyme in the regulation of ABA synthesis in higher plants (Tan *et al.*, 1997). By over expressing *AtNCED3 Arabidopsis* plants increased endogenous ABA level and promoted transcription of drought- and ABA-inducible genes. Also, mutants leaves have a reduction in transpiration rate and an improvement in drought tolerance. By contrast, drought-sensitive phenotype was observed by antisense suppression and disruption of *AtNCED3*. Those results indicated that in *Arabidopsis* the expression of *AtNCED3* plays a crucial role in ABA biosynthesis pathway under drought-stressed conditions (Iuchi *et al.*, 2001).

In *Arabidopsis*, *ENHANCED RESPONSE TO ABA 1 (ERA1)*, encodes the β -subunit of a farnesyl-transferase which involve in ABA signaling. Plants lacking *ERA1* activity have increased tolerance to drought, however are also severely compromised in yield. Wang *et al.*, (2005) used a drought-inducible promoter to drive the antisense expression of *ERA1* in order to have a conditional, reversible down-regulation of ABA, in both *Arabidopsis* and canola plants. Transgenic plants gave consistently

higher yields over the conventional varieties under water stress condition. However, there was no difference in performance between transgenic and controls in sufficient water conditions, demonstrating that this approach has no yield-drag (Wang *et al.*, 2005).

The *DREB* (dehydration responsive element binding protein) subfamily genes are important in the ABA-independent drought tolerant pathways that induce the expression of stress response genes (Shinozaki and Yamaguchi-Shinozaki, 2007). Over-expression of the *DREB1* native form and constitutively activation of *DREB2* form increased the tolerance of transgenic *Arabidopsis* plants to drought, high salinity and cold (Shinozaki and Yamaguchi-Shinozaki 2007). Although these genes were initially identified in *Arabidopsis* plants, their presence and role in stress tolerance have been reported in many other important crops, such as rice, tomato, barley, canola, maize, soybean, rye, wheat and maize, indicating that this is a conserved, universal stress defense mechanism in plants (Shinozaki and Yamaguchi-Shinozaki, 2007). This functional conservation makes the *DREB* genes important targets for crop improvement for drought tolerance through genetic engineering.

There are considerable challenges remain even after elucidating these genetic mechanisms underlying drought tolerance. When this comes to agricultural crops, they are subjected to variable levels of multiple stresses in field conditions that plant's response to multiple stresses cannot be inferred from the response to individual stress. Therefore, studies should be focus to a combination of stresses (Mittler, 2006). Hence, newly developed varieties should essential to study to multiple stresses, and to carry out extensive field studies in a large range of conditions that assess tolerance as absolute yield increases.

References

- Addison J.A., 1993. Persistence and non-target effects of *Bacillus thuringiensis* in soil: A review. *Can J For Res* 23, pp. 2329–2342.
- Alexander D., Goodman, R.M., Gut-Rella, M., Glascock, C., Weymann, K., Friedrich, L., Maddox, D., Ahl-Goy, P., Luntz, T. and Ward, E.S.C.H.E.R.I.C.H.I.A., 1993. Increased tolerance to two oomycete pathogens in transgenic tobacco expressing pathogenesis-related protein 1a. *PNAS*, 90(15): 7327-7331.
- Alfonso-Rubí, J., Ortego F., Castañera P., Carbonero P. and Díaz I., 2003. Transgenic expression of trypsin inhibitor CMe from barley in Indica and Japonica rice, confers resistance to the rice weevil *Sitophilus oryzae*. *Transgenic Res.*, 12: 2331.
- Altpeter F., Diaz I., McAuslane H., Gaddour K., Carbonero P. and Vasil I.K., 1999. Increased insect resistance in transgenic wheat stably expressing trypsin inhibitor CMe. *Mol. Breeding*, 5: 5363.
- Annadana S. *et al.*, 2002. Effects of cysteine protease inhibitors on oviposition rate

- of the western flower thrips *Frankliniella occidentalis*, *J Insect Physiol* 48: 701–706.
- Annadana S., Peters J., Gruden K., Schipper A., Outchkourov N.S., Beekwilder M.J., Udayakumar M. and Jongsma M.A., 2002. Transgenic tobacco resistant to a bacterial disease by the detoxification of a pathogenic toxin. *Molecular and General Genetics* 219: 492-494.
- BANR (Board on Agriculture and Natural Resources) 2000. Genetically modified pest protected plant: science and regulation. Washington: National Academy Press.
- Barbosa A.E., Albuquerque É.V., Silva, M.C., Souza D.S., Oliveira-Neto O.B., Valencia A., Rocha T.L. and Grossi-de-Sa M.F., 2010. Alpha-amylase inhibitor-1 gene from *Phaseolus vulgaris* expressed in *Coffea arabica* plants inhibits alpha-amylases from the coffee berry borer pest. *BMC Biotechnol*, 10: 44.
- Barry G., Kishore G., Padgett S., Taylor M., Kolacz K., Weldon M., Re D., Eichholtz D., Fincher K. and Hallas L., 1992. Inhibitors of amino acid biosynthesis: strategies for imparting glyphosate tolerance to crop plants In *Biosynthesis and Molecular Regulation of Amino Acids in Plants*. Ed. by Singh, B.K., Flores, H.E., Shannon, J.C. American Society of Plant Physiologists, Rockville, MD. 139-145.
- Baulcombe D. C., Saunders G. R., Bevan M. W., Mayo M. A., Harrison B. D., 1986. Expression of biologically-active viral satellite RNA from the nuclear genome of transformed plants. *Nature* 321: 446–449.
- Bayley C., Trolinder N., Ray, C., Morgan M., Quisenberry J.E. and Ow D.W., 1992. Engineering 2,4-D resistance into cotton. *Theor Appl Genet* 83: 645–649.
- Bravo A., Gill S.S. and Soberón M., 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon*, 49: 423–435.
- Broadway R.M. and Duffey S.S., 1986. Plant proteinase inhibitors: mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exiqua*. *Journal of Insect Physiology*, 32(10): 827-833.
- Broderic, N.A., Raffa K.F. and Handelsman J., 2006. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proceedings of the National Academy of Sciences of the United States of America*. 103: 15196–15199.
- Broderick N.A., Robinson C.J., McMahon M.D., Holt J., Handelsman J. and Raffa K.F., 2009 Contributions of gut bacteria to *Bacillus thuringiensis*-induced mortality vary across a range of Lepidoptera. *BMC Biology*, 7: 11.
- Brogliè K., Chet I., Holliday M., Cressman R., Biddle P., Knowlton S., et al. (1991). Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. *Science* 254 1194–1197.
- Buzayan J.M., Gerlach W.L. and Bruening G., 1986. Satellite tobacco ringspot virus RNA: A subset of the RNA sequence is sufficient for autolytic processing. *PNAS*, 83: 8859-8862.

- Cech T.M., 1986. The chemistry of self-splicing RNA and RNA enzymes. *Science* 236:1532- 1539.
- Cellier F., B. Zaccamer M.D. Morch A.L. Haenni and Tepfer M., 1990. Strategies for interfering in vivo with replication of tumip yellow mosaic virus. Page 123 in *Proceedings. Eighth International Congress of Virology, 26-30 August 1990, Berlin, Germany. International Union of Microbiological Societies, Free University of Berlin, Germany.*
- Chipman D., Barak Z.E. and Schloss J.V., 1998. Biosynthesis of 2-aceto-2-hydroxy acid; cetolactate synthases and acetohydroxyacid synthases. *Biochim Biophys Acta* 1385: 401-419.
- Collmer A., 1996. Bacterial avirulence proteins: where's the action? *Trends Plant Sci* 1:209-210.
- Comai L., Sen L.C. and Stalker D.M., 1983. An altered *aroA* gene product confers resistance to the herbicide glyphosate. *Science*, 221(4608): 370-371.
- Comai L., Facciotti D., Hiatt W. R., Thompson G., Rose R. E., and Stalker D. M., 1985. Expression in plants of a mutant *aroA* gene from *Salmonella typhimurium* confers tolerance to glyphosate. *Nature* 317, 741-745.
- Coombs J.J., Douches D.S., Li W., Grafius E.J. and Pett W.L., 2002. Combining engineered (Bt-cry3A) and natural resistance mechanisms in potato for control of Colorado potato beetle. *Journal of the American Society for Horticultural Science*, 127(1): 62-68.
- Crickmore N., Zeigler D.R., Feitelson J., Schnepf E.S.C.H.E.R.I.C.H.I.A., Van Rie J., Lereclus D., Baum J. and Dean D.H., 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews*, 62(3): 807-813.
- Crute I.R. and Pink D., 1996. Genetics and Utilization of Pathogen Resistance in Plants. *Plant Cell*. 8: 1747-1755.
- Dalmay T., Horsefield R., Braunstein T.H. and Baulcombe D.C., 2001. SDE3 encodes an RNA helicase required for post-transcriptional gene silencing in *Arabidopsis*. *The EMBO Journal*, 20(8): 2069-2077.
- Dasgupta I., Malathi V.G. and Mukherjee S.K., 2003. Genetic engineering for virus resistance. *Curr Sci*, 84: 341-354.
- Della-Cioppa G., Bauer S.C., Taylor M.L., Rochester D.E., Klein B.K., Shah D.M., Fraley R.T. and Kishore G.M., 1987. Targeting a herbicide-resistant enzyme from *Escherichia coli* to chloroplasts of higher plants. *Nature Biotechnology* 5: 579-584.
- Di R., Purcell V., Collins G.B. and Ghabrial S.A., 1996. Production of transgenic soybean lines expressing the bean pod mottle virus coat protein precursor gene. *Plant Cell Rep.* 15: 746-50.
- Dill G.M., Sammons R.D., Feng P.C., Kohn F., Kretzmer K., Mehrsheikh A., Bleeke

- M., Honegger J.L., Farmer D., Wright D. and Haupfear E.A., 2010. Glyphosate: discovery, development, applications, and properties. *Glyphosate Resistance in Crops and Weeds: History, Development, and Management*, John Wiley and Sons, Inc., Hoboken. 1-33.
- Dixon R.A. and Paiva N.L., 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell*, 7(7): 1085-1097.
- Duan X., Li X., Xue Q., Abo-El-Saad M., Xu D. and Wu R., 1996. Transgenic rice plants harboring an introduced potato proteinase inhibitor II gene are insect resistant. *Nat. Biotechnol.* 14: 494-498.
- Düring K., Porsch P., Fladung M. and Lörz H., 1993. Transgenic potato plants resistant to the phytopathogenic bacterium *Erwinia carotovora*. *The Plant Journal*, 3(4): 587-598.
- Vancouver Ellingboe, A. H. (1976). Genetics of host-parasite interactions. In *Physiological Plant Pathology. Encyclopedia of Plant Physiology*, 4: 761-778.
- Ellingboe A. H. (1982). Genetical aspects of active defence. In *Active Defence Mechanisms in Plants*, Edited by R. K. S. Wood. New York: Plenum Press. 179-192.
- Feitelson J.S., Payne J. and Kim L., 1992. *Bacillus thuringiensis*: insects and beyond. *Nature Biotechnology*, 10(3): 271-275.
- Ferré J., Real M.D., Van Rie J., Jansens S. and Peferoen M., 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. *PNAS*, 88(12): 5119-5123.
- Fillatti J.J., Haissig B., McCown B., Comai L. and Riemenschneider D., 1988. Development of glyphosate-tolerant *Populus* plants through expression of a mutant *aroA* gene from *Salmonella typhimurium*. In *Genetic manipulation of woody plants*. 243-249).
- Fillatti J.J., Kiser J., Rose R. and Comai L., 1987. Efficient transfer of a glyphosate tolerance gene into tomato using a binary *Agrobacterium tumefaciens* vector. *Nature Biotechnology*, 5(7): 726-730.
- Flor H. H., 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9: 275-298.
- Forster A.C. and Symons R.H., 1987. Self-cleavage of plus and minus RNAs of a virusoid and a structural model for the active sites. *Cell*, 49(2): 211-220.
- Fraley R. T., Rogers S.G., Horsch R.B., and Barry G.F., 1986. Gene transfer in plants: a tool for studying gene expression and plant development. In *Proc. Symposium of the Society for Developmental Biology*. L. Bogorad, ed. *Molecular Developmental Biology*. New York, USA: June 18-20, 1984. XIII + 164P. Alan R. Liss, Inc.: New York, USA
- Frischmuth T. and Stanley J., 1993, Strategies for the control of geminivirus diseases, *J. Sem. Virol.*, 4: 329-337.

- Funke T., Han H., Healy-Fried M.L., Fischer M. and Schönbrunn E., 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. *PNAS* 103(35): 13010–13015.
- Gatehouse A.M., Norton E., Davison, G.M., Babbé S.M., Newell C.A. and Gatehouse J.A., 1999. Digestive proteolytic activity in larvae of tomato moth, *Lacanobia oleracea*; effects of plant protease inhibitors in vitro and in vivo. *Journal of Insect Physiology*, 45(6): 545-558.
- Gatehouse A.M., Davison G.M., Newell C.A., Merryweather A., Hamilton W.D., Burgess E.P., Gilbert R.J. and Gatehouse J.A., 1997. Transgenic potato plants with enhanced resistance to the tomato moth, *Lacanobia oleracea*: growth room trials. *Molecular Breeding*, 3(1): 49-63.
- Gerlach W.L., Haseloff J.P., Young M.J. and Bruening G., 1990. Use of plant virus satellite RNA sequences to control gene expression. In *Viral Genes and Plant Pathogenesis*. 177-186.
- Goldman I.F., Arnold J., Carlton B.C., 1986. Selection for resistance to *Bacillus thuringiensis* subspecies *israelensis* in field and laboratory populations of the mosquito *Aedes aegypti*. *J. Invertebr. Pathol.* 47, 317–324.
- Gomase V.S. and Kale K.V., 2015. Information of Surface Accessibility of the Peptide Fragments of Coat Protein from Alfalfa mosaic virus (AMV) at the Physicochemical and Immunochemical Levels. *Drug Des*, 4(119): 2169-0138.
- Goryachkovsky T.N., Ananko E.A. and Kolpakov F.A., 2000. Gene network on plant interaction with pathogen organisms. BGRS'2000 Novosibirsk, Russia August 7-11, 2000. 188.
- Gronenberg B. 1990. Wheat dwarf virus vectors, a way to deliver chimaeric transposons to cereals. *Journal of Cellular Biochemistry (Supplement 14E 1990)*: 160.
- Grover A. and Gowthaman R., 2003. Strategies for development of fungus-resistant transgenic plants. *Current Science*, 84(3): 330-340.
- Halterman D.A. and Martin G.B., 1996. Signal recognition and transduction involved in plant disease resistance. *Essays in biochemistry*, 32: 87-99.
- Hammond S.M., Caudy A.A. and Hannon G.J., 2001. Post-transcriptional gene silencing by double-stranded RNA. *Nature Reviews Genetics*, 2(2): 110-119.
- Haseloff J. and Gerlach Wayne L., 1988. Simple RNA enzymes with new and highly specific endoribonuclease activities. *Nature*, 334: 585-591.
- He H., Wang Z. and Zhang Y., 2008. Monitoring Bt resistance in the field; China as a case study. In: Ferry N. & Gatehouse A.M.R., eds. *Environmental Impact of Genetically Modified/Novel Crops*. Wallingford, UK: CAB International.
- Hilder V.A., Gatehouse A.M., Sheerman S.E., Barker R.F. and Boulter D., 1987. A novel mechanism of insect resistance engineered into tobacco. *Nature*, 330(6144): 160-163.

- Hofte H. and Whiteley H.R., 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiology and Molecular Biology Reviews*, 53: 242–255.
- Hutchins C.J., Rathjen P.D., Forster A.C. and Symons R.H., 1986. Self-cleavage of plus and minus RNA transcripts of avocado sunblotch viroid. *Nucleic acids research*, 14(9): 3627-3640.
- Iannacone R., Grieco P.D. and Cellini F., 1997. Specific sequence modifications of a cry3B endotoxin gene result in high levels of expression and insect resistance. *Plant molecular biology*, 34(3): 485-496.
- Ignacimuthu S. and Prakash S., 2006. Agrobacterium-mediated transformation of chickpea with α -amylase inhibitor gene for insect resistance. *Journal of biosciences*, 31(3): 339-345.
- Iuchi S., Kobayashi M., Taji T., Naramoto M., Seki M., Kato T., Tabata S., Kakubari Y., Yamaguchi-Shinozaki K. and Shinozaki K., 2001. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *The Plant Journal*, 27(4): 325-333.
- Iulek J., Franco O.L., Silva M., Slivinski C.T., Bloch C., Rigden D.J. and de Sá M.F.G., 2000. Purification, biochemical characterisation and partial primary structure of a new α -amylase inhibitor from *Secale cereale* (rye). *The international journal of biochemistry & cell biology*, 32(11): 1195-1204.
- Jach G., Logemann S., Wolf G., Oppenheim A, Chet I., Schell J. and Logemann, J., 1992. Expression of a bacterial chitinase leads to improved resistance of transgenic tobacco plants against fungal infection. *Biopractice*, 1: 1-10.
- Jach G., Görnhardt B., Mundy J., Logemann J., Pinsdorf E., Leah R., Schell J. and Maas C., 1995. Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. *The Plant Journal*, 8(1): 97-109.
- James C., 2012. Global status of commercialized biotech/GM crops. Brief No. 44. Ithaca, NY, USA: ISAAA.
- James C., 2013. Global status of commercialized biotech/GM crops. Brief No. 46. Ithaca, NY, USA: ISAAA.
- Jansens S., Van Vliet A., Dickburt C., Buysse L., Piens C., Saey B., De Wulf A., Gossele V., Paez A., Göbel E. and Peferoen M., 1997. Transgenic corn expressing a Cry9C insecticidal protein from *Bacillus thuringiensis* protected from European corn borer damage. *Crop Science*, 37(5): 1616-1624.
- Jepson I., Holt D.C., Roussel V., Wright S.Y. and Greenland A.J., 1997. Transgenic plant analysis as a tool for the study of maize glutathione S-transferases. Regulation of enzymatic systems detoxifying xenobiotics in Plants. 313-323.
- Ketting R.F., Haverkamp T.H., van Luenen H.G. and Plasterk R.H., 1999. Mut-7 of *C. elegans*, required for transposon silencing and RNA interference, is a homolog

- of Werner syndrome helicase and RNaseD. *Cell*, 99(2): 133-141.
- Hammond-Kosack K.E. and Jones J.D., 1997. Plant disease resistance genes. *Annual review of plant biology*, 48(1), pp.575-607.
- Kim S.H. and Cech T.R., 1987. Three-dimensional model of the active site of the self-splicing rRNA precursor of *Tetrahymena*. *PNAS*, 84(24): 8788-8792.
- Kjemtrup S., Sampson K.S., Peele C.G., Nguyen L.V., Conkling M.A., Thompson, W.F. and Robertson, D., 1998. Gene silencing from plant DNA carried by a geminivirus. *The Plant Journal*, 14(1): 91-100.
- Klement Z., 1982. Hypersensitivity: In: Mount MS, Lacy GH (eds) *Phytopathogenic Prokaryotes 2*: 150-177.
- Kozziel M.G., Beland G.L., Bowman C., Carozzi N.B., Crenshaw R., Crossland L., Dawson J., Desai N., Hill M., Kadwell S. and Launis K., 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Nature Biotechnology*, 11(2): 194-200.
- Kunik T., Salomon R., Zamir D., Navot N., Zeidan M., Michelson I., Gafni Y. and Czosnek H., 1994. Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus. *Nature Biotechnology*, 12(5): 500-504.
- Li G., Xu X., Xing H., Zhu H. and Fan Q., 2005. Insect resistance to *Nilaparvata lugens* and *Cnaphalocrocis medinalis* in transgenic indica rice and the inheritance of *gna+* *sbt1* transgenes. *Pest management science*, 61(4): 390-396.
- Lichtenstein C.L. and Buck K.W., 1990. Expression of antisense RNA in transgenic tobacco plants confers resistance to geminivirus infection. *Molecular Strategies for Crop Improvement*. 17-24.
- Liu D.O.N.G., Raghobhama K.G., Hasegawa P.M. and Bressan R.A., 1994. Osmotin overexpression in potato delays development of disease symptoms. *Proceedings of the National Academy of Sciences*, 91(5): 1888-1892.
- Logemann J. D., Jach G., Tommerup H., Mundy J. D., and Schell J. P. 1992. Expression of a barley ribosome-inactivating protein leads to increased fungal protection in transgenic tobacco plants. *Bio/Technology* 10:305-308.
- Lorence A., Darszon A., Díaz C., Liévano A., Quintero R. and Bravo A., 1995. δ -Endotoxins induce cation channels in *Spodoptera frugiperda* brush border membranes in suspension and in planar lipid bilayers. *FEBS letters*, 360(3): 217-222.
- Lorito M., Woo S.L., D'ambrosio M., Harman G.E., Hayes C.K., Kubicek C.P. and Scala F., 1996. Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds. *MPMI-Molecular Plant Microbe Interactions*, 9(3): 206-213.
- Macrae T.C., Baur M.E., Boethel D.J., Fitzpatrick B.J., Gao A.G., Gamundi J.C., Harrison, L.A., Kabuye, V.T., Mcpherson, R.M., Miklos, J.A. and Paradise, M.S.,

2005. Laboratory and field evaluations of transgenic soybean exhibiting high-dose expression of a synthetic *Bacillus thuringiensis* cry1A gene for control of Lepidoptera. *Journal of economic entomology*, 98(2): 577-587.
- Mauch-Mani B. and Slusarenko A.J., 1996. Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *Peronospora parasitica*. *The Plant Cell*, 8(2): 203-212.
- McGaughey W.H., 1985 Insect resistance to the biological insecticide *Bacillus thuringiensis*. *Science* 229: 193-195.
- Mittler R., 2006. Abiotic stress, the field environment and stress combination. *Trends Plant Sci.* 11: 15-19.
- Morton R.L., Schroeder H.E., Bateman K.S., Chrispeels M.J., Armstrong E. and Higgins T.J., 2000. Bean α -amylase inhibitor 1 in transgenic peas (*Pisum sativum*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. *Proceedings of the National Academy of Sciences*, 97(8): 3820-3825.
- Mourrain P., Béclin C., Elmayan T., Feuerbach F., Godon C., Morel J.B., Jouette D., Lacombe A.M., Nikic S., Picault N. and Ré moué K., 2000. *Arabidopsis* SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell*, 101(5): 533-542.
- OECD 2007 Consensus document on safety information on transgenic plants expressing *Bacillus thuringiensis*-derived insect control proteins. No. 42. Paris Environment Directorate, Organisation for Economic Co-operation and Development.
- Oksman-Caldentey K.M. and Barz W.H. eds., 2002. *Plant biotechnology and transgenic plants* (Vol. 92). CRC press. Ou, S. H. 1985. *Rice Diseases*, Commonwealth Mycological Institute Publication, Kent, Surrey, UK., 280.
- Padgett S.R., Kolacz K.H., Delannay X., Re D.B., LaVallee B.J., Tinius C.N., Rhodes W.K., Otero Y.I., Barry G.F., Eichholtz D.A. and Peschke V.M., 1995. Development, identification, and characterization of a glyphosate-tolerant soybean line. *Crop science*, 35(5): 1451-1461.
- Plasterk R.H. and Ketting R.F., 2000. The silence of the genes. *Current opinion in genetics & development*, 10(5): 562-567.
- Powell K.S., Gatehouse A.M.R., Hilder V.A. and Gatehouse J.A., 1995. Antifeedant effects of plant lectins and an enzyme on the adult stage of the rice brown planthopper, *Nilaparvata lugens*. *Entomologia Experimentalis et Applicata*, 75(1): 51-59.
- Powell-Abel P., Nelson R.S., De B., Hoffmann N., Rogers S.G., Fraley R.T. and Beachy R.N., 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science*, 232, pp.738-744.
- Prody G.A., Bakos J.T., Buzayan J.M., Schneider I.R. and Bruening G., 1986. Autolytic

- processing of dimeric plant virus satellite RNA. *Science*, 231: 1577-1581.
- Punja Z.K., 2001. Genetic engineering of plants to enhance resistance to fungal pathogens—a review of progress and future prospects. *Canadian Journal of Plant Pathology*, 23(3): 216-235.
- Rahb, Y., Deraison C., Bonadé-Bottino M., Girard C., Nardon C. and Jouanin L., 2003. Effects of the cysteine protease inhibitor oryzacystatin (OC-I) on different aphids and reduced performance of *Myzus persicae* on OC-I expressing transgenic oilseed rape. *Plant science*, 164(4): 441-450.
- Reeck G.R., Kramer K.J., Baker J.E., Kanost M.R., Fabrick J.A. and Behnke C.A. 1997. Proteinase inhibitors and resistance of transgenic plants to insects. In: Carozzi N. & Koziel M., eds. *Advance in insect control: the role of transgenic plants*. 157-183.
- Ribeiro A.D.O., Pereira E.J.G., Galvan T.L., Picanco M.C., Picoli E.D.T., Silva D.D., Fari, M.G. and Otoni, W.C., 2006. Effect of eggplant transformed with oryzacystatin gene on *Myzus persicae* and *Macrosiphum euphorbiae*. *Journal of Applied Entomology*, 130(2): 84-90.
- Robertson J.M., Pharis R.P., Huang Y.Y., Reid D.M. and Yeung E.C., 1985. Drought-induced increases in abscisic acid levels in the root apex of sunflower. *Plant Physiology*, 79(4): 1086-1089.
- Ryan C.A., 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual review of phytopathology*, 28(1): 425-449.
- Saha P., Majumder P., Dutta I., Ray T., Roy S.C. and Das S., 2006. Transgenic rice expressing *Allium sativum* leaf lectin with enhanced resistance against sap-sucking insect pests. *Planta*, 223(6): 1329.
- Sahi C., Singh A., Blumwald E. and Grover A., 2006. Beyond osmolytes and transporters: novel plant salt-stress tolerance-related genes from transcriptional profiling data. *Physiologia Plantarum*, 127(1): 1-9.
- Sanyal I., Singh A.K., Kaushik M. and Amla D.V., 2005. Agrobacterium-mediated transformation of chickpea (*Cicer arietinum* L.) with *Bacillus thuringiensis* cry1Ac gene for resistance against pod borer insect *Helicoverpa armigera*. *Plant Science*, 168(4): 1135-1146.
- Schnepf H.E., Lee S., Dojillo J., Burmeister P., Fencil K., Morera L., Nygaard L., Narva K.E. and Wolt J.D., 2005. Characterization of Cry34/Cry35 binary insecticidal proteins from diverse *Bacillus thuringiensis* strain collections. *Applied and Environmental Microbiology*, 71(4): 1765-1774.
- Schünemann R., Knaak N. and Fiuza L.M., 2014. Mode of action and specificity of *Bacillus thuringiensis* toxins in the control of caterpillars and stink bugs in soybean culture. *ISRN microbiology*, 2014.
- Senthil-Kumar M. and Mysore K.S., 2013. Non-host resistance against bacterial pathogens: retrospectives and prospects. *Annual review of phytopathology*,

- 51, pp.407-427.
- Shade R.E., Schroeder H.E., Pueyo J.J., Tabe L.M., Murdock L.L., Higgins T.J.V. and Chrispeels M.J., 1994. Transgenic pea seeds expressing the α -amylase inhibitor of the common bean are resistant to bruchid beetles. *Nature Biotechnology*, 12(8): 793-796.
- Sharma H.C., Sharma K.K. and Crouch J.H., 2004. Genetic transformation of crops for insect resistance: potential and limitations. *Critical Reviews in Plant Sciences*, 23(1): 47-72.
- Shinozaki K. and Yamaguchi-Shinozaki K., 2007. Gene networks involved in drought stress response and tolerance. *Journal of experimental botany*, 58(2): 221-227.
- Smyth D.R., 1999. Gene silencing: plants and viruses fight it out. *Current biology*, 9(3): R79.
- Song X.X., and Wang S.M., 2001. Status and evaluation on the expression of cotton varieties in the production in China in the past 20 years. *Cotton Sci.*, 13:315-320.
- Steinrücken H.C., Schulz A., Amrhein N., Porter C.A. and Fraley R.T., 1986. Overproduction of 5-enolpyruvylshikimate-3-phosphate synthase in a glyphosate-tolerant *Petunia hybrida* cell line. *Archives of biochemistry and biophysics*, 244(1): 169-178.
- Strobl S., Maskos K., Betz M., Wiegand G., Huber R., Gomis-RuÈth F.X. and Glockshuber R., 1998. Crystal structure of yellow meal worm α -amylase at 1.64 Å resolution. *Journal of molecular biology*, 278(3): 617-628.
- Suslow T.V., Matsubara D., Jones J., Lee R. and Dunsmuir P., 1988. Effect of expression of bacterial chitinase on tobacco susceptibility to leaf brown spot. *Phytopathology*, 78(12): 1556.
- Tabashnik B.E., Cushing N.L., Finson N. and Johnson M.W., 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 83(5): 1671-1676.
- Tabashnik B.E., Finson N. and Johnson M.W., 1991. Managing Resistance to *Bacillus thuringiensis*: Lessons from the Diamondback Moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 84(1): 49-55.
- Tabei Y., Kitade S., Nishizawa Y., Kikuchi N., Kayano T., Hibi T. and Akutsu K., 1998. Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*). *Plant Cell Reports*, 17(3): 159-164.
- Tan S., Evans R. and Singh B., 2006. Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops. *Amino acids*, 30(2): 195-204.
- Tohidfar M., Ghareyazie B., Mosavi M., Yazdani S. and Golabchian R., 2008. Agrobacterium-mediated transformation of cotton (*Gossypium hirsutum*) using a synthetic cry1Ab gene for enhanced resistance against *Heliothis armigera*. *Iranian Journal of Biotechnology*, 6(3): 164-173.

- Tohidfar M., Zare N., Jouzani G.S. and Eftekhari S.M., 2013. Agrobacterium-mediated transformation of alfalfa (*Medicago sativa*) using a synthetic cry3a gene to enhance resistance against alfalfa weevil. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 113(2): 227-235.
- Tougou M., Furutani N., Yamagishi N., Shizukawa Y., Takahata Y. and Hidaka S., 2006. Development of resistant transgenic soybeans with inverted repeat-coat protein genes of soybean dwarf virus. *Plant cell reports*, 25(11): 1213-1218.
- Umezawa T., Fujita M., Fujita Y., Yamaguchi-Shinozaki K. and Shinozaki K., 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current opinion in biotechnology*, 17(2): 113-122.
- Uno Y., Furihata T., Abe H., Yoshida R., Shinozaki K. and Yamaguchi-Shinozaki K., 2000. Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *Proceedings of the National Academy of Sciences*, 97(21): 11632-11637.
- USDA 2000. APHISPPQPRRA Biotechnology Authorizations. Permits notifications and determinations of nonregulated status (as of 6/30/2000), <http://www.aphis.usda.gov> (Biotechnology section)
- Vaeck M., Reynaerts A., Höfte H., Jansens S., De Beuckeleer M., Dean C., Zabeau M., Montagu M.V. and Leemans J., 1987. Transgenic plants protected from insect attack. *Nature*, 328: 33-37.
- Vandenborre G., Smagghe G. and Van Damme E.J., 2011. Plant lectins as defense proteins against phytophagous insects. *Phytochemistry*, 72(13): 1538-1550.
- Vivian A. and Gibbon M.J., 1997. Avirulence genes in plant-pathogenic bacteria: signals or weapons?. *Microbiology*, 143(3): 693-704.
- Wang Y., Ying J., Kuzma M., Chalifoux M., Sample A., McArthur C., Uchacz T., Sarvas C., Wan J., Dennis D.T. and McCourt P., 2005. Molecular tailoring of farnesylation for plant drought tolerance and yield protection. *The Plant Journal*, 43(3): 413-424.
- Whalon M.E., and McGaughey W.H., 1993a. Insect resistance to *Bacillus thuringiensis*. In: L Kim ed. *Advanced Engineered Pesticides*. New York: Marcel Dekker, 215-232.
- Whalon M.E., Miller D.L., Hollingworth R.M., Grafius E.J. and Miller J.R., 1993b. Selection of a Colorado potato beetle (*Coleoptera: Chrysomelidae*) strain resistant to *Bacillus thuringiensis*. *Journal of Economic Entomology*, 86(2): 226-233.
- Williams S., Friedrich L., Dincher S., Carozzi N., Kessmann H., Ward E. and Rylas J., 1992. Chemical regulation of *Bacillus thuringiensis* δ -endotoxin expression in transgenic plants. *Nature Biotechnology*, 10(5): 540-543.
- Wu G., Shortt B.J., Lawrence E.B., Levine E.B., Fitzsimmons K.C. and Shah D.M.,

1995. Disease resistance conferred by expression of a gene encoding H₂O₂-generating glucose oxidase in transgenic potato plants. *The Plant Cell*, 7(9): 1357-1368.
- Zhang X., Candas M., Griko N.B., Taussig R. and Bulla L.A., 2006. A mechanism of cell death involving an adenylyl cyclase/PKA signaling pathway is induced by the Cry1Ab toxin of *Bacillus thuringiensis*. *Proceedings of the National Academy of Sciences*, 103(26): 9897-9902.
- Zhou H., Arrowsmith J.W., Fromm M.E., Hironaka C.M., Taylor M.L., Rodriguez, D., Pajeau, M.E., Brown, S.M., Santino, C.G. and Fry, J.E., 1995. Glyphosate-tolerant CP4 and GOX genes as a selectable marker in wheat transformation. *Plant Cell Reports*, 15(3): 159-163.
- Zou C., Zhang Z., Wu S. and Osterman J.C., 1998. Molecular cloning and characterization of a rabbit eIF2C protein. *Gene*, 211(2): 187-194.