



Transgenic Breeding in Crop Plants for Food Security

***Corresponding Author(s): IC Mohanty**

Associate Professor, Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar, India.
Email: icmohanty1@rediffmail.com

Abstract

The higher crop production and productivity through advanced crop improvement programme can sustain adequate food supply for the growing population. Crop improvement can be ensured through suitable combinations of genes by conventional plant breeding supplemented by molecular techniques called transgenic breeding. Higher yield or potential crop yield is seldom realised due to various kinds of stresses the crop plants suffer from. Identification of stress-related genes and their transfer to suitable host crop plants helps in effective stress management so that the gap between expected yield and actual yield can be minimized. In the current study, valuable information on stress related genes has been reviewed for stress management in crop plants for increasing crop production through transgenic breeding. The practical application of transgenic breeding in the farmers' field and release of transgenics for commercial use has also been reviewed.

Received: Aug 01, 2021

Accepted: Sep 16, 2021

Published Online: Sep 18, 2021

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: <http://meddocsonline.org/>

Copyright: © Mohanty IC (2021). *This Article is distributed under the terms of Creative Commons Attribution 4.0 International License*

Keywords: Stress factors; Plant stress response; Transgenic breeding; Crop improvement; Commercial Transgenics.

Introduction

Domestication of various plant species, a practice that started some 10,000 years back, considered to be the earliest form of plant breeding and plant biotechnology led the foundation for modern crop improvement programme which aims at putting of continuous effort for increasing crop production and productivity. Early attempts for crop improvement by traditional breeding through natural selection, mutation breeding and artificial breeding followed by selection has made several modifications in plant architecture and genetic background that made the crop plants tolerant to various stress factors, thus en-

hancing the crop yield. Nevertheless, the traditional breeding has its own limitations like cross-incompatibility barrier, imprecise selection after gene introgression in cross-compatible species. Desired characters available in unrelated species cannot be transferred by conventional breeding through normal hybridization. The modern molecular breeding methods like rDNA technology is a powerful method of precise delivery of a desired trait through DNA segment from any organism to the genomic background of a cultivated species which is not at all possible through traditional breeding.



The potential yield of a crop plant can be enhanced in many diverse ways of modifying basic functions of a plant, such as growth rate, photosynthesis, plant architecture, nutrient uptake and metabolism, ratio of above-ground to below ground biomass, transition to flowering, seed set, harvest index and source-sink relationship etc. However, the potential yield is seldom realized in the prevailing field conditions due to the various stress conditions. According to Boyer [1], most crops are at best only reaching 20 percent of the genetic potential for yield due to various stress conditions in the field. Therefore, there is urgent need to develop crops where the yield gap can be bridged to increase the crop production and productivity of a crop.

Stress factors and plant responses

Stress is generally perceived as the reaction of a living system to any unfavourable environmental conditions that, depending on their severity and period of occurrence, may trigger considerable alterations in the system [2-4]. Those various environmental factors which bring about functional and structural alterations to the extent that stress of the plants induces reduced growth, physiological adaptation, decreased bio-production, or some combination of these changes are known as stressors or stress factors [3,5]. Nilsen and Orcutt [3] have categorised different sources of stress factors mainly into two as abiotic (drought, salinity, air pollution, radiation, high temperature or freezing etc.) and biotic stress (pathogen, competition with other organisms) which may affect the plants in many ways. When the plant experiences primary stress due to drought, cold, heat, salinity or any kind of chemical pollution, it develops in to further different kind of stresses called secondary stress like oxidative stress due to reactive oxygen species and osmotic stress which may lead to damage to functional & structural proteins and membranes, disruption of osmotic and ionic homeostasis. Subsequently, various physiological and molecular activities by the organisms like perception of the stress, signal transduction, induction of transcription factors and activation of related genes act in a cascade manner help retrieve functional & structural protection of proteins & membranes and cellular homeostasis leading to stress resistance/tolerance [6]. Stress-responsive transcription leads to expression of regulatory genes which in turn might start cascades of regulation by transcription factors as late responses to environmental stresses that manifests in the form of the metabolomic alterations e.g. the generations of compatible compounds like phytoalexins, antioxidants, cry protectants, protein protectants [7] and proteomic changes like production of novel defence proteins and protein chaperones and increased expression of constitutive defence proteins [8,9].

Abiotic stress related genes

The toleration to abiotic stresses in crop plants depend on either preclusion or palliation of cellular damage, the resumption of growth and the retrieval of homeostatic conditions. Understanding the molecular and physiological basis of these toleration factors will lead to identification and engineering of candidate genes for tolerance in plants to abiotic stress.

Osmotic stress related genes

There is formation of compatible solutes as a result of osmotic stress caused due to primary abiotic stress which allow the plant to adapt that particular stress situation. A number of such biosynthates like amino acids & amines and sugars & sugar alcohols have been identified in plants which ameliorate abiotic stress-tolerance in crop plants.

Aminoacids

Proline accumulation in plant cells during osmotic stress is a metabolic response of plants where the enzyme, 1-pyrroline-5-carboxylate synthetase (P5CS), plays a major role in this pathway [10]. Proline is considered to act as a molecular chaperone, as a scavenger of Reactive Oxygen Species (ROS), store of carbon and nitrogen and even as a signal for other adaptive responses to abiotic and biotic stresses [11]. Therefore, it is of definite interest to develop drought tolerant crop plants in various crop species.

Amines

Glycine-betaine is an amine that is found in a wide variety of microorganisms, plants and animals naturally [12]. In general, it is biosynthesized due to oxidation of choline in a two-step process via a toxic intermediate, betaine aldehyde where two important enzymes viz. Choline Monooxygenase (CMO) and Betaine Aldehyde Dehydrogenase (BADH) play major role [13]. The synthesis of these two enzymes found mostly in the chloroplast stroma increases with increase in salt stress.

Sugars

Trehalose (D-glucopyranosyl-D-glucopyranoside) generally acts as a compatible solute in the stabilization of cellular activities under abiotic stress in invertebrates, bacteria and fungi. But, in case of plants it is synthesized to a detectable level only in "resurrection plants" tolerant to desiccation [14].

Sugar alcohol

The Mannitol as a sugar-alcohol is found in various organisms [15,16] and its biosynthesis in higher plants is regulated by three important enzymes viz. Man-6-phosphate (Man6P) isomerase, Man6P dehydrogenase, and mannitol-1-phosphatase (M1Pase). Mannitol, as a compatible solute, takes part in osmoregulation, storage and regeneration of reducing power in both algae [16] and plants [17].

Osmotin genes

Osmotin, belonging to PR-5 protein family gets accumulated as a result of osmotic stress and maintains cell osmotic potential. It takes part in osmotic adjustment by the cells either by facilitating the compartmentation or accumulation of solutes or by participating in structural or metabolic changes during osmotic adjustment.

Oxidative stress-related genes

Reactive Oxygen Species (ROS) like superoxide, the hydroxyl radical, hydrogen peroxide (H_2O_2) and singlet oxygen are known to be generated as a result of oxidative stress. The plant defense systems usually operate either by blocking the formation of active oxygen species generated due to stress or by scavenging them after their formation. The low molecular mass scavengers such as phenolic compounds, ascorbic acid & the tri-peptide glutathione and enzymes such as catalase, super oxide dismutase (SOD) and peroxidase are thought to be the components of oxidative defence system in plants [18,19].

Regulatory and signalling genes

There are a number of regulatory and signalling genes, which interact among themselves and regulate the transcription of related genes into mRNA that translates into either to a structural protein or to an enzyme which further controls the expression

of some other genes. These proteins which are of regulatory or activator in nature are known as transcription factors that are the main players in cascades or regulatory networks. A no. of transcription activators have been identified which regulates the expression of many downstream genes related to different types of abiotic stresses.

DREB/CBF

One of the greater understood transcription activator, dehydration-responsive element binding factor (DREB)/C-repeat (CRT) binding factor (CBF) was isolated from *Arabidopsis thaliana*, which regulates the expression of gene related to dehydration caused due to salinity, drought and cold stress [20,21]. The two classes of DREBs isolated are- 1. DREB1 expression up-regulated during cold stress, and 2. DREB2 expression up-regulated in response to drought and salinity [22]. Another regulatory gene, HARDY (HRD) belonging to the BREB/CRB family, has been reported to induce increased mesophyll cell layer, dense root system, and enhanced tolerance to salinity and drought [23].

Protein kinase

Various members of Protein kinases viz. 1. Mitogen-activated protein kinase (MAPK), 2. Calcineurin B-like protein-interacting protein kinase (CIPK), and 3. Calcium-dependent protein kinase (CDPK) have been suggested to be involved in drought tolerance. Sanders et al. [24] have reported that Ca^{2+} cytosolic levels mediated by the members of the CDPK family increase rapidly in plant cells in response to environmental stress, including salinity and drought.

NAC proteins

A group of three major transcription factors having conserved domains known as NAC proteins viz. 1. NAM (no apical meristem), 2. ATAF (Arabidopsis transcription activation factor) and 3. CUC (cup-shaped cotyledon) have been reported to be related with abiotic stresses [25]. They regulate various stress signalling pathways which may include interplay of phytohormones.

LEA proteins

LEA (late embryogenesis abundant) proteins are produced in abundance during seed development, comprising up to 4% of cellular proteins [26] and prevent protein aggregation during desiccation or water stress [27]. However, many LEA proteins are also induced by cold or osmotic stress which protect cellular or molecular structures with proposed mechanisms like ion sequestration, hydration buffering, direct protection of other proteins & membranes, and re-naturation of unfolded proteins during water stress [28].

Aquaporins

Aquaporins are pore-forming integral membrane proteins characterized by six transmembrane helices that selectively allow water or other small uncharged molecules to pass along the osmotic gradient [29]. The aquaporins regulating cellular water transport had also been reported by Knepper [30].

Biotic stress and stress response

Almost all the crops particularly cash crops suffer from huge economic losses caused by biotic stress. The production of various antimicrobial proteins and phytoalexins as part of plant responses to different biological stresses has been reported by several group of researchers [31-33]. Plant responds to biotic

stress in two ways-1. Systemic acquired resistance (SAR) involving ethylene and salicylic acid and 2. Induced systemic resistance (ISR) involving jasmonic acid as signalling molecules.

Genes for insect resistance

Various organisms usually produce insecticidal molecules which are effective against insects and innocuous to man and other organisms and those can be deployed in crop plants in a sustainable and safe fashion [34].

Resistance genes isolated from micro-organisms

Bacillus thuringiensis (Bt) toxin

The insecticidal crystalline proteins (ICP) produced by *B. thuringiensis* during sporulation are highly toxic to insect even at very low concentrations. The genes for ICPs known as Bt toxin genes (*Cry* genes) more than 100 in number are grouped into distinct families (about 40) designated as *Cry* 1.....to *Cry* 40, on the basis of size and sequence similarities.

The important insect resistant genes isolated from other micro-organisms are -

i. **Cholesterol oxidase** isolated from *Streptomyces* has been transferred to tobacco and the transgenic plants show resistance to boll weevil larvae.

ii. **Isopentenyl transferase** gene isolated from *Agrobacterium tumefaciens* has been transferred to tobacco & tomato and the transgenic plants show resistance to tobacco hornworm and peach potato aphid respectively.

Resistance genes isolated from higher plants

The following list of insecticidal proteins have been isolated from higher plants and are being used to develop transgenic crops resistant to insects.

Proteinase inhibitors

Proteinase inhibitors (PI's) are induced in plants in response to different stress conditions like insect & pathogen attack, mechanical wounding and UV exposure. They inhibit the gut proteinases of the insect as a result the insects suffer from growth retardation and subsequently death, thus, minimizing the crop damage.

α -Amylase inhibitors

The α - amylase secreted by insect for its starch digestion can be blocked by α -amylase inhibitor due to which the insect is killed. Coleoptera type of insects have been controlled by inserting α -Amylase inhibitor gene (α -AI-Pv) isolated from bean in case of tobacco.

Plant lectins

Because of affinity for specific carbohydrate moieties Lectins bind to glycoproteins of the insect midgut as a result digestive processes and nutrient assimilation are disrupted leading to death of insect. The tobacco and potato plants transformed with a lectin gene isolated from snow drop (*Galanthus nivalis*) have shown resistance to aphids and the tomato moth (*Lacania oleracea*) respectively.

Insect chitinases

Insect chitinases are the insecticidal protein that target chitin found in exo-skeletal and gut lining of insects as insoluble

structural polysaccharide which protects the insect against water loss and abrasive agents. Therefore, expressing the chitinases produced by insects themselves interfere with chitin affecting the growth and moulting of insects by which insects can be controlled.

Genes for disease resistance

Anti-fungal molecules like proteins and toxins (Pathogenesis related proteins):

There are proteins known as pathogenesis related proteins which get accumulated during fungal infections in plants and break chitin and glucon in the cell walls of fungal pathogen. Major classes of such PR proteins like PR-1, PR- 2 (β 1-3 glucanase), PR-3 (Chitinases), PR-4 (Hevein like), PR-5 (Thaumatin like and Osmotin) etc. can be exploited to develop crop resistance to pathogens.

Anti-microbial proteins

Plants and other organisms may contain cysteine rich anti-microbial protein such as lectins, defensins, thionins, lysozyme, polygalacturonase inhibitors etc. which can inactivate the ribosomes and hence, exhibit resistance to pathogens.

Phytoalexins

Phytoalexins are secondary metabolites having antimicrobial properties synthesized by the plant and provide resistance to diseases in response to an infection. Resveratrol is one of a phytoalexin synthesized by the enzyme resveratrol synthase also known as stilbenesynthase or STS.

Transgenic breeding

Transgenic breeding or genetic engineering is the most advanced method of plant breeding where genes from any organism can be transferred to a suitable crop species which can confer adaptation to different stresses and improved productivity. Such transgenic plants can be released as an improved cultivar or can be used as a donor or germplasm as new source of variation in breeding programs.

Transgenic crop development

The development of transgenic crops involves identification of a suitable gene, manipulation and incorporation into a desired organism for getting a novel phenotype. Understanding biochemical and physiological mechanisms of action, regulation

of gene expression and safety of gene and gene product will ensure successful development of a transgenic plant. However, the process of developing a transgenic plant involves a number of steps. Briefly these are- 1. Identification of a source organism from which desired gene will be isolated, 2. Isolation of genetic material from the source organism, 3. Fragmentation of DNA, cloning and development of gene library, 4. Screening of the library to get the gene of interest, 5. Suitable manipulation of the gene and development of gene-construct with desired promoter, 6. Genetic transformation using *Agrobacterium tumefaciens* or particle bombardment followed by invitro regeneration, 7. Detection of transgene and subsequently its expression and environmental safety analysis.

Crops improved through transgenic breeding and impact of transgenics

The first commercially successful transgenic crop was the soybean, which is known as Roundup Ready resistant to glyphosate herbicide, now also sold generically as glyphosate. Later, Bt corn was produced with insecticidal gene, isolated from *Bacillus thuringiensis* which showed resistance some pests. Both of these innovations in crop improvement programme saved lot of time and money. These transgenic traits have since been added to many other major crops including cotton, corn, rice, canola, alfalfa and sugar beets (Table-1). The area under Biotech crop has increased manifold almost 113-fold since 1996, with an estimated area of 2.5 billion hectares recently, thus, proving that biotechnology is the fastest accepted crop technology in the world. Now, average yields for soybeans, corn and other popular GMO crops have increased since that introduction by 25 percent in corn yields [35].

Ariel and Riesgo [36] in their meta-analysis on all GMO crops concluded, "On average, GM technology adoption has reduced chemical pesticide use by 37%, increased crop yields by 22%, and increased farmer profits by 68%." Brooks and Barfoot [37] reported that farmers are growing more without using additional land due to transgenic crops. As per the estimate, maintaining global production levels at the 2014 levels would have required additional plantings of 8.9 million ha of corn, 7.5 million ha of soybeans, 3.7 million ha of cotton and 0.6 million ha of canola, without this transgenic technology in 2014. This total area requirement is equivalent to 12% of the arable land in the US, or 14% of the cropping area in China or 33% of the arable land in Brazil.

20.i	Potato - <i>Solanum tuberosum</i> L.	Modified starch/ carbohydrate	gbss (antisense fragment)	<i>Solanum tuberosum</i>	production of granule-bound starch synthase (GBSS) enzyme is suppressed by a gene silencing mechanism	reduces the levels of amylose and increases the levels of amylopectin in starch granules	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	BASF	Starch Potato & Innate® Cultivate
20.ii			gbss (antisense fragment)	<i>Solanum tuberosum</i>	-do-	-do-	<i>Agrobacterium tumefaciens</i> -mediated plant transformation		Amflora™
20.iii	Lowered Free Asparagine, Reduced Black Spot, Lowered Reducing Sugars, Foliar Late Blight Resistance		asn1	<i>Solanum tuberosum</i>	double stranded RNA	generates dsRNA to down regulate Asn1 transcripts which lowers asparagine formation	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	J.R. Simplot Co.	Innate® Hibernata
			ppo5	<i>Solanum verrucosum</i>	ds RNA	generates dsRNA to down regulate Ppo5 transcripts which lowers black spot bruise development			
			Rpi-vnt1	<i>Solanum venturii</i>	late blight resistance protein	provides resistance to potato late blight			
			PhL	<i>Solanum tuberosum</i>	double stranded RNA	generates dsRNA to down regulate PhL transcripts which lowers reducing sugars			
			R1	<i>Solanum tuberosum</i>	double stranded RNA	generates dsRNA to down regulate R1 transcripts which lowers reducing sugars			
			VInv	<i>Solanum tuberosum</i>	double stranded RNA	Down regulates VInv transcripts which lowers reducing sugars			
21.	Rice - <i>Oryza sativa</i> L.	Mannose metabolism . Enhanced Protein A Content	crt1	<i>Pantoea ananatis</i>	phytoene desaturase enzyme CRT1	catalyzes the conversion of 15-cis-phytoene to all-trans-lycopene	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	International Rice Research Institute	Golden Rice
			psy1	<i>Zea mays</i>	phytoene synthase ZmPSY1	converts geranylgeranyl diphosphate into phytoene, and acts upstream of CRT1 in the carotenoid biosynthesis pathway			
			pml *	<i>Escherichia coli</i>	Phosphomannose Isomerase (PMI) enzyme	metabolizes mannose and allows positive selection for recovery of transformed plants			
22.i	Soybean - <i>Glycine max</i> L.	Sulfonylurea herbicide tolerance , Modified oil/fatty acid	gm-hra *	<i>Glycine max</i>	modified acetolactate synthase (ALS) enzyme	provides tolerance to sulfonylurea – based herbicides	Particle bombardment of plant cells or tissue	DuPont (Pioneer Hi-Bred International Inc.)	Treus™, Plenish™
			gm-fad2-1 (partial sequence)	<i>Glycine max</i>	no functional enzyme is produced (expression of the endogenous fad2-1 gene encoding omega-6 desaturase enzyme was suppressed by the partial gm-fad2-1 gene fragment)	blocks the formation of linoleic acid from oleic acid (by silencing the fad2-1 gene) and allows accumulation of oleic acid in the seed			
22.ii		Glyphosate herbicide tolerance , Modified oil/fatty acid	fatb1-A (sense and antisense segments)	<i>Glycine max</i>	no functional enzyme (FATB) produced	reduces the levels of saturated fatty acids and increases the levels of 18:1 oleic acid	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	Monsanto Company (including fully and partly owned companies)	Vistive Gold™

15.ii		Phytase production	phyA	<i>Aspergillus niger</i> var. van Tieghem	3-phytase enzyme	Breakdown of plant phytates which bind phosphorus and makes the latter available to monogastric animals	Agrobacterium tumefaciens-mediated plant transformation	BASF	Phytaseed™ Canada
16.i	Carnation - Dianthus caryophyllus	Sulfonylurea herbicide tolerance, Modified flower color	dfr	<i>Petunia hybrida</i>	dihydroflavonol-4-reductase (DFR) hydroxylase enzyme	catalyzes the production of the blue-coloured anthocyanin pigment delphinidin and its derivatives	Agrobacterium tumefaciens-mediated plant transformation	Florigene Pty Ltd. (Australia)	Moondust™
			hfl (f3'5'h)	<i>Petunia hybrida</i>	Flavonoid 3',5'-hydroxylase (F3'5'H) enzyme	-do-			
			surB *	<i>Nicotiana tabacum</i>	herbicide tolerant acetolactate synthase (ALS) enzyme	provides tolerance to sulfonylurea herbicides and other acetolactate synthase (ALS) inhibiting herbicides			
16.ii		Sulfonylurea herbicide tolerance, Modified flower color	dfr	<i>Petunia hybrida</i>	dihydroflavonol-4-reductase (DFR) hydroxylase enzyme	catalyzes the production of the blue-coloured anthocyanin pigment delphinidin and its derivatives	Agrobacterium tumefaciens-mediated plant transformation	Florigene Pty Ltd. (Australia)	Moonshadow™
			bp40 (f3'5'h)	<i>Viola wittrockiana</i>	Flavonoid 3',5'-hydroxylase (F3'5'H) enzyme	-do-			
			surB *	<i>Nicotiana tabacum</i>	herbicide tolerant acetolactate synthase (ALS) enzyme	confers tolerance to sulfonylurea herbicides and other acetolactate synthase (ALS) inhibiting herbicides			
16.iii		Sulfonylurea herbicide tolerance, Modified flower color	dfr	<i>Petunia hybrida</i>	dihydroflavonol-4-reductase (DFR) hydroxylase enzyme	anthocyanin pigment delphinidin and its derivatives	Agrobacterium tumefaciens-mediated plant transformation	Florigene Pty Ltd. (Australia)	Moonlite™
			hfl (f3'5'h)	<i>Petunia hybrida</i>	Flavonoid 3',5'-hydroxylase (F3'5'H) enzyme	anthocyanin pigment delphinidin and its derivatives			
			surB *	<i>Nicotiana tabacum</i>	herbicide tolerant acetolactate synthase (ALS) enzyme	provides tolerance to sulfonylurea herbicides and other acetolactate synthase (ALS) inhibiting herbicides			
17.	Cotton - Gossypium hirsutum L.	Low Gossypol	dCS	<i>Gossypium hirsutum L.</i>	dsRNA that suppresses the expression of d-cadinene synthase gene involved in gossypol biosynthesis, thru RNAi pathway	silence the endogenous dCS genes	Agrobacterium tumefaciens-mediated plant transformation	Texas A&M AgriLife Research University	Event Code : TAM-66274-5
18.i	Maize - Zea mays L.	Modified alpha amylase, Mannose metabolism	amy797E	synthetic gene derived from <i>Thermococcus</i> spp.	thermostable alpha-amylase enzyme	increases bioethanol production by enhancing the thermostability of amylase used in degrading starch	Agrobacterium tumefaciens-mediated plant transformation	Syngenta	Enogen™
18.ii		Modified amino acid	cordapA	<i>Corynebacterium glutamicum</i>	dihydrodipicolinate synthase enzyme	increases the production of amino acid lysine	Microparticle bombardment of plant cells or tissue	Renessen LLC (Netherlands)	Mavera™ Maize
19.	Pineapple - Ananas comosus	Delayed ripening/senesescence, Modified fruit color	Psy	Tangerine (<i>Citrus reticulata</i>)	phytoene	increases lycopene and/or beta-carotene levels	Agrobacterium tumefaciens-mediated plant transformation	Del Monte Fresh Produce Company	Rosé
			b-Lyc	<i>Ananas comosus</i>	gamma-carotene	increases lycopene accumulation using RNAi technology			
			e-Lyc	<i>Ananas comosus</i>	delta-carotene	-do-			
			acc	<i>Ananas comosus</i>	1-aminocyclopropane-1-carboxylic acid synthase	involved in catalyzing the penultimate step in ethylene biosynthesis			

8.iv	(Singular) Insect Resistance	cry1Ac	<i>Bacillus thuringiensis</i> subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin	-do-	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	Monsanto Company (including fully and partly owned companies)	Bollgard™ Cotton
8.v	(Stacked) Herbicide Tolerance + Insect Resistance	cry1Ab	<i>Bacillus thuringiensis</i> subsp. kurstaki	Cry1Ab delta-endotoxin	-do-	Cross hybridization and selection involving transgenic donor(s)	Bayer CropScience (including fully and partly owned companies)	TwinLink™ Cotton
9.i	Maize - <i>Zea mays</i> L.	cry2Ae	<i>Bacillus thuringiensis</i> subsp. Dakota	Cry2Ae delta-endotoxin	-do-			
		bar	<i>Streptomyces hygroscopicus</i>	phosphinothricin N-acetyltransferase (PAT) enzyme		eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation		
		ecry3.1Ab	synthetic form of Cry3A gene and Cry1Ab gene from <i>Bacillus thuringiensis</i>	chimeric (Cry3A-Cry1Ab) delta endotoxin protein		provides resistance to coleopteran and lepidopteran insects by selectively damaging their midgut lining	Syngenta	Agrisure® Duracade™
		pmi *	<i>Escherichia coli</i>	Phosphomannose isomerase (PMI) enzyme		metabolizes mannose and allows positive selection for recovery of transformed plants		
9.ii	(Stacked) Herbicide Tolerance + Insect Resistance	cry34Ab1	<i>Bacillus thuringiensis</i> strain P5149B1	Cry34Ab1 delta-endotoxin		Provides resistance to coleopteran insects particularly corn rootworm by selectively damaging their midgut lining	Dow AgroSciences LLC and DuPont (Pioneer Hi-Bred International Inc.)	Herculex™ RW
		cry35Ab1	<i>Bacillus thuringiensis</i> strain P5149B1	Cry35Ab1 delta-endotoxin	-do-			
		pat	<i>Streptomyces viridochromogenes</i>	phosphinothricin N-acetyltransferase (PAT) enzyme		eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation		
9.iii	(Stacked) Herbicide Tolerance + Insect Resistance	cry1Ab	<i>Bacillus thuringiensis</i> subsp. kurstaki	Cry1Ab delta-endotoxin		provides resistance to lepidopteran insects by selectively damaging their midgut lining	Syngenta	Bt10
		pat	<i>Streptomyces viridochromogenes</i>	phosphinothricin N-acetyltransferase (PAT) enzyme		eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation		
		bla *	<i>Escherichia coli</i>	beta lactamase enzyme		detoxifies beta lactam antibiotics such as ampicillin		
9.iv	(Stacked) Herbicide Tolerance + Insect Resistance	cry9C	<i>Bacillus thuringiensis</i> subsp. toloworthi strain BTS02618A	Cry9C delta endotoxin		provides resistance to lepidopteran insects by selectively damaging their midgut lining	Bayer CropScience (including fully and partly owned companies)	Starlink™ Maize
		bar	<i>Streptomyces hygroscopicus</i>	phosphinothricin N-acetyltransferase (PAT) enzyme		eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation		
		bla *	<i>Escherichia coli</i>	beta lactamase enzyme		detoxifies beta lactam antibiotics such as ampicillin		
9.v	(Stacked) Herbicide Tolerance + Insect Resistance	cry1Ab	<i>Bacillus thuringiensis</i> subsp. kurstaki	Cry1Ab delta-endotoxin		provides resistance to lepidopteran insects by selectively damaging their midgut lining	Syngenta	NaturGard Knock-Out™, Maximizer™

7.iv	Lowered free asparagine	(Stacked) Disease Resistance + Modified Product Quality	asn1	<i>Solanum tuberosum</i>	double stranded RNA	Generates dsRNA to down regulate Asn1 transcripts that reduces asparagine formation	<i>Agrobacterium tumefaciens-mediated plant transformation</i>	J.R. Simplot Co.	Innate® Acclimate
	Reduced black spot		ppo5	<i>Solanum verrucosum</i>	ds RNA	Generates dsRNA to down regulate Ppo5 transcripts and lowers black spot bruise development			
	Foliar late blight resistance		Rpi-vnt1	<i>Solanum venturii</i>	late blight resistance protein	Provides resistance to potato late blight			
	Lowered Reducing sugars		PhL	<i>Solanum tuberosum</i>	dsRNA	generates dsRNA to down regulate PhL transcripts which lowers reducing sugars			
	Lowered Reducing sugars		R1	<i>Solanum tuberosum</i>	dsRNA	generates dsRNA to down regulate R1 transcripts that lowers reducing sugars			
	Lowered Reducing sugars		Vinv	<i>Solanum tuberosum</i>	dsRNA	downregulates Vinv transcripts that lowers reducing sugars			
8.i	Cotton - <i>Gossypium hirsutum</i> L.	Insect resistance (Stacked)	cry1F	<i>Bacillus thuringiensis</i> var. aizawai	Cry1F delta-endotoxin	provides resistance to lepidopteran insects by selectively damaging their midgut lining	Cross hybridization and selection involving transgenic donor(s)	Dow AgroSciences LLC	WideStrike™ Cotton
			cry1Ac	<i>Bacillus thuringiensis</i> subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin	Provides resistance to lepidopteran insects by selectively damaging their midgut lining			
8.ii	(Stacked) Herbicide Tolerance + Insect Resistance	Herbicide resistance (Glufosinate)	pat (syn) *	synthetic form of pat gene derived from <i>Streptomyces viridochromogenes</i> strain Tu 494	phosphinothricin N-acetyltransferase (PAT) enzyme	removes herbicidal activity of glufosinate (phosphinothricin) by acetylation		Monsanto Company and Dow AgroSciences LLC	WideStrike™ Roundup Ready™ Cotton
			cp4 epsps (aroA:CP4)	<i>Agrobacterium tumefaciens</i> strain CP4	herbicide tolerant form of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme	reduces binding affinity for glyphosate, provides tolerance to glyphosate	Cross hybridization and selection involving transgenic donor(s)		
			cry1F	<i>Bacillus thuringiensis</i> var. aizawai	Cry1F delta-endotoxin	provides resistance to lepidopteran insects by selectively damaging their midgut lining			
			cry1Ac	<i>Bacillus thuringiensis</i> subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin	provides resistance to lepidopteran insects by selectively damaging their midgut lining			
8.iii	(Stacked) Herbicide Tolerance + Insect Resistance		bar	<i>Streptomyces hygroscopicus</i>	phosphinothricin N-acetyltransferase (PAT) enzyme	eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation		Monsanto Company (including fully and partly owned companies)	BXN™ Plus Bollgard™ Cotton
			bxn	<i>Klebsiella pneumoniae</i> subsp. <i>Ozaenae</i>	nitrilase enzyme	eliminates herbicidal activity of oxynil herbicides (eg. bromoxynil)	<i>Agrobacterium tumefaciens-mediated plant transformation</i>		
			cry1Ac	<i>Bacillus thuringiensis</i> subsp. Kurstaki strain HD73	Cry1Ac delta-endotoxin	provides resistance to lepidopteran insects by selectively damaging their midgut lining			

Table 1: Commercial Transgenics in the farmers' field (Source: <http://www.isaaa.org>).

Sl. No.	Name of the Crop	Trait Modified	Gene Introduced	Gene Source	Product	Function	Method of genetic transformation	Name of the organization developed	Name of the Transgenic
1	Maize - <i>Zea mays L</i>	Drought tolerance	cspB	<i>Bacillus subtilis</i>	cold shock protein B	Maintaining normal cellular activity under drought stress	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	Monsanto Company and BASF	Genuity® DroughtGard™
2	Soybean - <i>Glycine max L</i>	Drought tolerance	Hahb-4	<i>Helianthus annuus</i>	transcription factor Hahb-4	Regulates the genes associated with dehydration stress	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	Verdeca	Verdeca HB4 Soybean
3	Sugarcane - <i>Saccharum sp</i>	Drought tolerance	EcBeta	<i>Escherichia coli</i>	choline dehydrogenase	Synthesis of glycine betaine conferring water stress-tolerance	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	PT Perkebunan Nusantara XI (Persero)	Event Code: NXI-1T
4	Bean - <i>Phaseolus vulgaris</i>	Disease Resistance : Viral resistance	ac1 (sense and anti-sense)	Bean-Golden Mosaic Virus (BGMV)	antisense and sense RNA of viral replication protein (Rep)	no production of functional viral replication protein	Particle-bombardment of plant cells or tissue	EMBRAPA (Brazil)	Event Code: EMB-PV051-1
5.i	Papaya - <i>Carica papaya</i>	Viral resistance	prsv_cp	Papaya ringspot virus (PRSV)	coat protein (CP) of the papaya ringspot virus (PRSV)	conferring resistance to papaya ringspot virus (PRSV) through the mechanism of "pathogen-derived resistance"	Particle bombardment of plant cells or tissue	Cornell University and University of Hawaii	Rainbow, SunUp
5.ii		Viral resistance	prsv_rep	Papaya ringspot virus (PRSV)	replicase domain of the papaya ringspot virus (PRSV)	conferring resistance to papaya ringspot virus (PRSV) through gene silencing mechanism	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	South China Agricultural University	Huanong No. 1
6.	Plum - <i>Prunus domestica</i>	Viral resistance	ppv_cp	Plum pox virus (PPV)	coat protein of plum pox virus (PPV)	provides resistance to plum pox virus (PPV) through the mechanism of "pathogen-derived resistance"	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	USDA- Agricultural Research Service	ARS-PLMC5-6 (Event code)
7.i	Potato - <i>Solanum tuberosum L.</i>	Viral resistance + Insecticidal resistance	cry3A	<i>Bacillus thuringiensis</i> subsp. tenebrionis	cry3A delta endotoxin	conferring resistance to coleopteran insects by selectively damaging their midgut lining	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	Monsanto Company (including fully and partly owned companies)	Hi-Lite NewLeaf™ Y potato
7.ii		Viral resistance + Insecticidal resistance (Staked)	pvv_cp	Potato Virus Y (PVY)	coat protein of the potato virus Y (PVY)	provides resistance to potato virus Y (PVY) through "pathogen-derived resistance" mechanism			
7.ii		Viral resistance + Insecticidal resistance (Staked)	cry3A	<i>Bacillus thuringiensis</i> subsp. tenebrionis	cry3A delta endotoxin	Provides resistance to coleopteran insects by selectively damaging their midgut lining	<i>Agrobacterium tumefaciens</i> -mediated plant transformation	Monsanto Company (including fully and partly owned companies)	New Leaf™ Y Russet Burbank potato
7.iii		Viral resistance + Insecticidal resistance	cry3A	Potato Virus Y (PVY)	coat protein of the potato virus Y (PVY)	provides resistance against potato virus Y (PVY) through the mechanism of "pathogen-derived resistance"			
		Viral resistance + Insecticidal resistance	plrv_orf1	<i>Bacillus thuringiensis</i> subsp. tenebrionis	cry3A delta endotoxin	Provides resistance to coleopteran insects by selectively damaging their midgut lining			New Leaf™ Plus Russet Burbank potato
			plrv_orf2	Potato Leaf Roll Virus (PLRV)	putative helicase domain of the potato leaf roll virus (PLRV)	provides resistance to potato leaf roll virus (PLRV) through gene silencing			
				Potato Leaf Roll Virus (PLRV)	putative helicase domain of the potato leaf roll virus (PLRV)	Provides resistance to potato leaf roll virus (PLRV) through gene silencing			

Conclusion

The success of transgenic breeding depends on complete elucidation of plant response to different stresses, underlying mechanisms and identification of related genes which can meet the challenging task of producing sustainable higher crop production under changing climates and stressful environmental conditions. A number of novel genes for different types of stress conditions so far have been identified which have been used to develop transgenic crops with commercial value having direct contributions to food quality, clean environment, and pharmaceutical production in various crops. Thus the transgenic technology has been proved to be a powerful technology for producing designer crops as per the need of the society.

References

- Boyer JS. Plant productivity and the environment. *Science*. 1982; 218: 443-448.
- Göring H. Reaktionen der Pflanzen auf extreme physikalische und chemische Umweltbedingungen. *Umwelt-Stress. Wiss. Beitr. Martin-Luther-Univ, Wittenberg*. 1982; 35: 152-160.
- Nilsen ET, Orcutt DM. *The Physiology of Plants under Stress: Abiotic Factors*. New York, John Wiley & Sons. 1996; 689.
- Godbold DL. Stress concepts and forest trees. *Chemosphere*. 1998; 36: 859-864.
- Grime JP. Stress, competition, resource dynamics and vegetation processes. *Plant Adaptation to Environmental Stress*. London, Chapman & Hall. 1993; 45-65.
- Krauss G. *Biochemistry of signal transduction and regulation*. Wiley, New York. 2001.
- Bohnert HJ, Shen B. Transformation and compatible solutes. *Sci. Hortic*. 1999; 78: 237-260.
- Cushman JC, Bohnert HJ. Genomic approaches to plant stress tolerance. *Curr Opin Plant Biol*. 2000; 3: 117-124.
- Grover A, Sahi C, Sanan N, Grover A. Taming abiotic stress in plants through genetic engineering, current strategies and perspective. *Plant Sci*. 1999; 143:101-111.
- Delaung AJ, Verma DPS. Proline Biosynthesis and Osmoregulation in Plants. *The Plant Journal*. 1993; 2: 215-223.
- Verbruggen N, Hermans C. Proline accumulation in Plants a review. *Aminoacids (Vienna)*. 2008; 35: 753-759.
- Rhodes D, Hanson AD. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology*. 1993; 44: 357-384.
- Weigel P, Weretilnyk EA, Hanson AD. Betaine aldehyde oxidation by spinach chloroplasts. *Plant Physiology*. 1986; 82: 753-759.
- Goddijn OJM, Van Dun K. Trehalose metabolism in plants. *Trends in Plant Science*. 1999; 4: 315-319.
- Stoop JM, Mooibroek H. Cloning and characterization of NADP-mannitol dehydrogenase cDNA from the button mushroom, *Agaricus bisporus*, and its expression in response to NaCl stress. *Appl Environ Microbiol*. 1998; 64: 4689-4696.
- Kremer BP, Kirst GO. Biosynthesis of photosynthates and taxonomy of algae. *Z. Naturforsch.37C*. 1982; 761-771.
- Loescher WH, Tyson RH, Everard JD, Redgwell RJ, Bielecki RL. Mannitol synthesis in higher plants. *Plant Physiol*. 1992; 98: 1396-1402.
- Scandalios JC. Response of plant antioxidant defense genes to environmental stress. *Adv Gen*. 1990; 28: 1-40.
- Bowler C, Van Montagu M, Inze D. Superoxide dismutase and stress tolerance. *Annu Rev Plant Physiol Plant Mo1 Biol*. 1992; 43:83-116.
- Yamaguchi-Shinozaki K, Shinozaki K. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *The Plant Cell*. 1994; 6:251-264.
- Yamaguchi-Shinozaki K, Shinozaki K. Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends in Plant Science*. 2005; 10: 88-94.
- Sagare DB, Mohanty IC. Development of Moisture Stress Tolerant Brinjal cv. Utkal Anushree (*Solanum melongena* L.) using Agrobacterium Mediated Gene Transformation. *Journal of Agricultural Science*. 2012; 4: 141-148.
- Karaba A. Improvement of Water Use Efficiency in Rice and Tomato Using Arabidopsis Wax Biosynthetic Genes and Transcription Factors, Wageningen. 2007.
- Sanders D, Pelloux J, Brownlee C, Harper JF. Calcium at the crossroads of signalling. *The Plant Cell*. 2002; 14: S401-417.
- Riechmann JL, Heard J, Martin G, Reuber L, Jiang CZ, et al. Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science*. 2000; 290: 2105-2110.
- Welin BV, Olson A, Nylander M, Palva ET. Characterisation and differential expression of DHN/LEA/RAB-like genes during cold-acclimation and drought stress in Arabidopsis thaliana. *Plant Mol Biol*. 1994; 26:131-144.
- Goyal K, Walton LJ, Browne JA, Burnell AM, Tunnacliffe A. Molecular anhydrobiology: Identifying molecules implicated in invertebrate anhydrobiosis. *Integr Comp Biol*. 2005; 45: 702-709.
- Cuming AC. LEA proteins. In *Seed Proteins*. Kluwer Academic Publishers, Dordrecht. 1999; 753-780.
- Tyerman SD, Niemietz CM, Bramley H. Plant aquaporins: multifunctional water and solute channels with expanding roles. *Plant Cell Environ*. 2002; 25: 173-194.
- Knepper MA. The aquaporin family of molecular water channels. *Proc Natl Acad Sci USA*. 1994; 91: 6255-6258.
- Slusarenko A, Fraser RSS, Van Loon LC. The mechanisms of resistance to plant disease. Kluwer, Dordrecht. 2000.
- Broekaert WF, Terras FRG, Cammue BPA. Induced and preformed antimicrobial proteins. In: *The mechanisms of resistance to plant disease*. 2000: 371-477.
- Mansfield JW. Antimicrobial compounds and resistance. In: *The mechanisms of resistance to plant disease*. 2000: 325-370.
- Sharma HC, Sharma KK, Seetharama N, Romero O. Prospects for using transgenic resistance to insects in crop improvement. *Electronic Journal of Biotechnology*. 2000; 3: 76-95.
- Pellegrino E, Bedini S, Nuti M, Ercoli L. Impact of genetically engineered maize on agronomic, environmental and toxicological traits: a meta-analysis of 21 years of field data. *Nature*. 2018; 15.
- Areal FJ, Riesgo L, Rodríguez-Cerezo E. Economic and agronomic impact of commercialized GM crops: a meta-analysis. *Journal of Agricultural Science*. 2013; 151: 7-33.
- Graham Brookes G, Barfoot P. Global income and production impacts of using GM crop technology 1996-2014. *GM Crops and Food*. 2016: 38-77.

38. Kishore PBK, Hong Z, Miao GH, Hu CAA, Verma DPS. Overexpression of Δ -1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.* 1995; 108: 1387-1394.