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A Review On: Impact of Genetic Engineering in Biotic Stresses Resistance Crop Breeding

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ABSTRACT

Genetic engineering is recombinant DNA technology that involves artificial addition, deletion or rearrangement of sequences of bases in DNA to alter form and function of organism. It complement plant breeding efforts by increasing the diversity of genes and germplasm available for incorporation into crops and by shortening the time required for the production of new varieties and hybrids. As the conventional breeding is restricted to sexually compatible crop species, time intensive and random process the genetic engineering is an alternative method to develop promising varieties with higher resistance to biotic stresses. Genetic engineering facilitates development of biotic stress resistant crops by expressing bacterial δ-endotoxins and vegetative insecticidal proteins, plant genes like lectins, protease inhibitors, RNA interference and genome editing through CRISPR Cas9. *Bt*-crops (maize, cotton Tobacco, Soyabean and etc), *Bacillus thuringiensis* (*BT*) are insect-resistant crops and the most outstanding achievements through genetic engineering of insecticidal protein coding genes from soil bacterium *B. thuringiensis.* Several studies indicated that genetically modified crops have reduced pesticide quantity by 37% and pesticide cost by 39% and on average crop yields increased by 21%. Transgenic lines of banana and tomato have showed resistance to *Banana Xanthomonas wilt* (BXW) and Fusarium wilt, respectively. In summary, Genetic engineering has played pivotal role in developing biotic resistance cultivars and cultivation area of these crops is growing fast each year, which indicates understanding and applying this new technologies offer more effective solutions against evolving biotic stress.

Keywords- Biotic Stresses, Genetic Engineering, Resistance.

I. INTRODUCTION

Genetic engineering is recombinant DNA technology that involves artificial addition, deletion or rearrangement of sequences of bases in DNA in order to alter the observable form and function of an organism. Though conventional breeding has remained a mainstay of agricultural farming practices, it is time intensive, restricted within species and random process to assort the genes [1]. In line with this, genetic engineering approach has been demonstrated to provide enormous options for the selection of the resistance genes from different sources to introduce them into plants to provide resistance against different biotic stresses [2]. The method complement plant breeding efforts by increasing the diversity of genes and germplasm available for incorporation into crops and shortening the time required for the production of new varieties and hybrids [3]. Combating various types of biotic stresses is the foundation and crux of sustainable agriculture.

Biotic stress is damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, insects, weeds, and cultivated or native plants [4]. The degree of biotic stresses imposed on a plant depends on geography, climate, host plant and its ability to resist. Agricultural research mainly focuses on, due to the large economic losses it caused to cash crops. The relationship between biotic stress and plant yield affects economic decisions as well as practical development. The impacts of biotic injury on crop yield will impacts population dynamics, plant-stressor co-evolution, and ecosystem nutrient cycling [5].

The devastation to crops and the societies that depend on these crops caused by viruses, fungi, bacteria, nematodes and herbivorous insects are well documented and among others the cause of the massive migration of Irish farmers to North America in the middle of the 19th century after the attack of potato fields by *Phytophtora infestans* killing or displacing 25% of the Irish population [6]. Destruction of banana plantations by *Fusarium oxysporum f. sp. cubense* also called the Panama disease [7]. Crops yield are reduced about 25% worldwide due to diseases and insects infestation [8]. This crop has been devastating from Fusarium wilt, Club root, Soft rot, Black rot are the importance diseases [9]. Devastating wheat stem rust called Ug99 is not onlythe major threat in Africa but also globally, since 90% of the wheat varieties grown worldwide are susceptible to this pathogen [10]. Crop yield losses due to insects are estimated between 30% and 60% in Africa [11]. Pesticides and fungicides are widely used to (generally) successfully control the yield reductions caused by biotic stress, but their harmful effects on environment and human health are now largely debated. In Italy study was conducted on impact of genetically modified crop and on average crop yields increased by 21%. These yield increases are not due to higher genetic yield potential, but to more effective pest control and thus lower crop damage. At the same time, these crops have reduced pesticide quantity by 37% and pesticide cost by 39% [12]. Hence, the objective of this

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paper is to explore and discuss the contributions of genetic engineering technology in developing biotic stresses resistance crop varieties.

II. GENETIC ENGINEERING IN BIOTIC STRESS MANAGEMENTS

Insect-Pest Resistance

The major classes of insect that cause crop damage are the orders Lepidoptera (Butterflies and moths), Diptera (flies and moths), Orthoptera (grasshoppers and crickets), Homoptera (aphids) and Coleopteran (beetles) [13]. Genetic engineering plays a pivotal role in conferring resistance against these insects [14]. It is being exploited to introduce specific DNA sequences or genes into crop plants through Agrobacterium-mediated transformation or particle bombardment for insect control [15].

Transgenic plants producing insecticidal Cry proteins (ICP) have made a tremendous impact on the successful development of insect resistance crop varieties. Bt is a potent insecticide containing crystal protein endotoxin produced by some strains of soil bacterium *B. thuringiensis*. The *Bt*-crystal (Cry) insecticidal protein (δ-endotoxin) genes are highly selective and represent class of numerous proteins with insecticidal action on larvae from various insect orders: *Cry1* and *Cry2* are toxic for lepidopteran pests, *Cry2A* for lepidopterans and dipteran pests, and *Cry3* for coleopteran pests [16]. *Bt* genes encoding insecticidal *Cry* proteins have been transferred to relevant crops to confer protection against their most important insect pests. *Cry* proteins once ingested by the insect are solubilized in the mid-gut and are then cleaved there by digestive proteases. Some of the resulting polypeptides are able to bind to mid-gut epithelial cell receptors resulting in cell lysis and finally insect death [17]. [18] and [19] pointed that resistance to insect pests was manifested mainly when Bt toxin producing ICP genes under the control of tissue specific or constitutive promoters and introduced in different crop species, including maize, sweet potato, cotton and tomato. 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 [20]. Most commercially planted *Bt* cotton contains *cry1Ac* or a fusion gene of *cry1Ac* and *cry1Ab* [20]. *Bt* corn is designed to control corn pests such as the European corn borer, corn earworm, and southwestern corn borer, and Bt cotton effectively controls cotton pests such as the tobacco budworm, cotton bollworm, and pink bollworm [21]. In India reports have suggested that the level of insecticide being used for a particular type of insect-resistant cotton (Bt cotton) was up to two thirds less than what would normally be used on this crop [12]. In 2010, Bt-maize was grown on 39 million hectares, an increase of 3.0 million hectares, or a year-over year growth rate of 10% [22].

Table1: Transgenic crops carrying *Bt* genes for insect resistance

S. n	Target Crop	Trans gene	Target Insect	References
1	Cotton	cry2AX1	H.armigera	[23]
2	Cotton	cry2Ab	Lepidopten pest	[19]
		cry1F	H.armigera, S.litura	
		cry1AC		
3	Sweet potato	cry1Aa	S. litura	[24]
4	Cotton	cry1AC	S. litura	[25]
		cry2Ab		
5	Soyabean	cry 8 like	Coleopteran-Holtrichia panallele	[26]
6	Cotton	cry2AX	H.armigera	[27]
7	Pigeon pea	cry2Aa	pod borer-H.armigera	[28]
8	Tomato	cry1Ac	Tuta Absoluta - tomato leaf miner	[29]
9	Chickpea	cryIIAa	Pod borer	[30]
10	Rice	cry2A	Leaf folder	[31]
11	Pigeon pea	cry1AC	H.armigera	[32]
		cry2Aa		
12	Pigeon pea	cry2Aa	H.armigera	[33]
13	Cotton	cry1Ab	Heliothis	[34]

Source: [35]

Lectins are carbohydrate-binding proteins and novel defense gene which enhance insect resistance crop breeding. Transgenic rice expressing *Allium sativum* leaf agglutinin and Galanthus nivalis lectin (GNA), showed insect resistance against major sap sucking pests including brown planthopper, white backed planthopper and green leafhopper [35]. [36] stated that expression of GNA gene in potato conferred resistance to aphids and in comparison, to non-transgenic plants. [37] reported that lentil lectin (LL) and Chickpea protease inhibitor (CPPI) genes were transformed into *Brassica juncea* and showed enhanced resistance to sap sucking pest, i.e., aphids. Around 69% of aphid population was reduced and 100% mortality of *Sclerotium litura* was observed within 96 h [38].

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RNA interference (RNAi) which is the process of sequence-specific suppression of gene expression and it is insecticidal strategy offers new dimensions for environment-friendly insect pest management in plants [39]. Double-stranded RNA (dsRNA) commonly used for interference of specific gene silencing through genetic modifications in plants for developing pathogen resistance. [40] stated that spraying of dsRNAs in maize, triggered the RNAi mechanism to initiate gene knockdown in piercing, sucking and stem borer insects and enhanced insect mortality rates. [41] also reported *dvvgr* and *dvbol* genes silencing in maize resulted in reduction of insect fecundity, minimal larval feeding and reduction in insect reproduction of western corn rootworm. In potato, through RNAi approach *EcR* gene (Ecdysone receptor) enhanced resistance against Colorado potato beetle (CPB) with an insect mortality of 15–80% and larval weight was reduced [42].

Clustered regularly interspaced short Palindromic repeats (CRISPR-Cas9) mediated genome editing is latest approach to develop insect resistance crop varieties. Cas9 is a monomeric RNA guided DNA endonuclease which contains two domains such as RuvC and HNH nucleases, which cleaves non-complementary and complementary DNA strands respectively, leading to formation of blunted in target DNA and subsequently disrupts function of a gene through formation of frame shift in the targeted region [43]. [44] reported the CRISPR/Cas9 induced mutation in two β-1- 3 *glucanase* genes in barley negatively affected aphid, growth and diminished the host preference in barely. CRISPR/Cas9 tool has been employed for knockout of many insect genes including *H. armigera, S. exigua* etc. Knockout of two ABC transporters, *PxABCC2* and *PxABCC3* in lepidopteran pest *Plutella xylostella* through CRISPR/Cas9 tool, resulted higher level of resistance to *cry1Ac* protoxin compared to susceptible strains [45].

III. DISEASES RESISTANCE

Modern agriculture must provide sufficient nutrients to feed the world's growing population through tackling crop loss challenges due to disease [46]. [47] reported bacterial and fungal pathogens reduce crop yields by about 15% and viruses reduce yields by 3%. Genetic engineering can make possible to save crops in the face of virulent disease epidemics, crops that may be integral to food security, sources of farmer income, or culturally important dietary components and also reduce farmers' dependence on pest-control products [48]. [49] have been reported that transgenic crop or plants in disease resistance are more important for genes not able to fix in conventional breeding and for crops that their breeding status is lagged.

[46] reported various genes like *chitinase*, *glucanase*, *osmotin*, *defensin*, etc. are being transferred into various horticultural crops world over for imparting resistance against bacterial and fungal diseases. Horticultural crops like potato and banana their breeding is slow down, the pathogens that attack these crops adapt the condition and devastate the crops which need use of transgenic approach, where many genes are pyramided for durable resistance. [50] expressed *plant ferredoxin like protein* (*Pf1p*) gene under the control of *CaMV35S* promoter in transgenic banana cv. to develop resistance against *Banana Xanthomonas wilt* (BXW) disease. Accordingly, 67% of the transgenic lines were found resistant to BXW and did not show any disease symptoms. [51] developed transgenic tomato plants over-expressing a wheat *chitinase* gene, *chi194*, under the control of maize *ubiquitin 1* promoter. The transgenic tomato lines showing higher expression of *chitinase* activity were found to be highly resistant to Fusarium wilt disease of tomato caused by *Fusarium oxysporum f.* sp. *Lycopersici*. [52] transferred a *Trichoderma-endochitinase* gene into guava (*Psidium guajava*) for resistance of Guava wilt disease caused by a soil borne fungus *Fusarium oxysporum f.* sp. *psidii*.

The resistance-gene *Rxo1* from maize was successfully introduced into rice and conferred resistance against bacterial streak disease caused by *Xanthomonas oryzae* [53]. Recently, a plant ferrodoxin like protein (PFLP) was transferred to Arabidopsis. Expression of PFLP protein enhanced resistance to bacterial disease. PFLP is a photosynthetic type ferredoxin with an N-terminal signal peptide for chloroplast localization. Presence of PFLP in transgenic plants confers resistance against bacterial disease; however, the relationship still remains unclear [54].

One of the most devastating fungal diseases that threaten the members of *Solanacea*, especially potatoes, is *Phytophthora infestans* also known as the late blight. To overcome this infection, several strategies using biotechnology-driven approaches to confer resistance to potato varieties have been proposed. In this regard, several R-genes (resistance) have been identified and isolated from various sources [55]. The *LpiO*gene, among the 54 tested effectors, was selected to stimulate innate immunity of Solanum species. Following the hypersensitive responses (HR) caused by *LpiO*, the source of the R gene *Rpi-blb1* was identified. The transient co-expression of *LpiO* (as effector) and *Rpi-blb1* (as resistance gene) in Nicotiana benthamiana led to rapid identification of *Rpi-sto1* and *Rpi-pta1* as resistant genes to late blight [56]. In another study, a stacking of three broad spectrum potato R-genes (*Rpi*), *Rpi-sto1* (*Solanum stoloniferum*), *Rpi-vnt1.1* (Solanum venturii) and *Rpi-blb3* (*Solanum bulbocastanum*) was transformed into susceptible cultivar and near 4% of the transformed plants showed HR against pathogenic effects of Phytophtora [57]. Fusarium head blight (FHB) is an important disease in wheat that may lead to contamination of the yielded products with mycotoxins (thrichothecene and deoxynivalenol-DON). Food contamination with DON is a risk for human and animal health. Recently, a *L3* gene (N-terminal fragment of yeast ribosomal protein) was

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transferred to wheat and the transgenic plants showed resistance to Fusarium disease and improved level of DON in transgenic wheat kernel [58].

Plant viruses cause significant economic losses worldwide [59]. Coat protein-mediated resistance to viruses has been one of the successes of plant genetic engineering and several major crop plants have been engineered to resist important viral pathogens. Expressing the *Tobacco mosaic virus* (TMV) coat protein in *Nicotiana tabacum* plants delayed the onset of symptoms on the transgenic plants when subsequently challenged by TMV. Besides, potato event HLMT15-15 tolerant to PYV (Potato Y Virus) or potato event RBMT21-350 resistant to PLRV (Potato Leaf Roll Virus) was produced [60]. [61] produced transgenic tobacco expressing defective Cucumber Mosaic Virus (CMV) replicase-derived dsRNA which was highly resistance. There are also reports on resistance to virus in transgenic plants mediated by a defective movement protein (MP) of virus [62]. Over expression of SpCas9 and artificially designed guide RNAs targeting various regions of *Tomato yellow leaf curl virus* (TYLCV) conferred resistance to the virus in *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*) [63]; [64].

IV. RESISTANCE TO HERBICIDES

Herbicide resistance (HR) is the predominant trait of cultivated GM crops and will remain so in the near future. HR traits are used on > 80% of the estimated 134 million hectares of transgenic crops grown annually in 25 countries [65]; [22]. There are different types of herbicides for different species of weeds some of them are glyphosate, glufosinate, synthesis auxin (2, 4-D), Acetyl coenzyme A carboxylase inhibitor, ALS inhibitor acetolactate syntheses (ALS; EC 2.2.1.6) [66]. GM crops resistant to the broad-spectrum herbicides glyphosate and glufosinate have first been cultivated commercially in the 1990s [67]. Glyphosate-tolerant maize, soybean, canola and cotton are the most abundant among crops genetically engineered for herbicide resistance. Glyphosate strongly competes with the substrate phosphoenol pyruvate (PEP) at the EPSPS enzyme-binding site in the chloroplast, resulting in the inhibition of the shikimate pathway [68]. A gene for a glyphosate insensitive EPSPS with enzymatic characteristics similar to plant EPSPS was isolated from common soil bacterium, *Agrobacterium tumefaciens* strain CP4 [69]. This *cp4 epsps* gene has been used to develop GR soybeans, cotton, corn, canola, alfalfa, bentgrass, and sugar beet [70].

The *bar gene* from bacteria strain *Streptomyces hygroscopicus* encodes a phosphinothricin acetyl transferase (PAT) that acetylates the free NH2 groups of phosphinothricin (PPT), the component of herbicides, thereby inactivating its herbicide activity. So, a transgenic line encoding PAT becomes resistant like the sweet potato expressing the *bar gene* [71]. Glyphosate oxidoreductase (GOX) from *Ochrobactrum anthropi strain* LBAA and the *pat* gene, homologues to *bar*, from *Streptomyces viridochromogenes* which encodes N-acetyltransferases are two other genes that can inactivate glyphosate and glufosinate, respectively [66].

A GmGSTU gene from soybean was transferred to tobacco. The GmGSTU4 is an isoenzyme which has catalytic activity for diphenylether herbicide fluorodifen/alachlor [72]. Recently, an imidazolinone resistance (IR) XA17 gene was introduced into maize. Transgenic lines showed resistance to imazaquin and nicosulfuron herbicides. Another mechanism that deactivates glyphosate into a nontoxic N-acetylglyphosate is by introducing the glyphosate N-acetyltransferase (Gat) from *Bacillus licheniformis* to plant [73].

In the US, the most often stated reasons for the adoption of HR crops were improved and simplified weed control, less labor and fuel cost, no-till planting/planting flexibility, yield increase, extended time window for spraying, and in some cases decreased pesticide input [74]. Overall herbicide use in HR crops has increased: From 1998 to 2013, the increase in amounts (kg/ha) of active ingredient (a.i.) in HR soybean was 64%, compared to 19% in conventional soybean [75]. Also, the use of herbicide resistance crop like soybean, maize, and cotton led to an increased use herbicide in the US from 1996 - 2011, compared to non-herbicide crops.

Whereas glyphosate resistance crops have been very successful, the evolution of glyphosate resistance weeds was faster and more widespread than many expected [66]. As a result, the next wave of technologies will combine resistance to glyphosate and other herbicides to provide growers with more herbicide options.

Table2: Transgenic Herbicide-Resistant Cotton, Corn and Soybeans

Crop	Resistance trait	Trait gene	Trait designation	First sales
Cotton	glyphosate	*cp4 cpsps*	MON1445	1996
		two cp4 cpsps	MON88913	2006
		Zm-2mcpsps	GHB614	2009
	Glufosinate	bar	LLCotton25	2005
Corn	glyphosate	three modified zm-mcpsps	GA21	1998
		two cp4 cpsps	NK603	2001
	Glufosinate	pat	T14, T25	1996

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Soybean	glyphosate	cp4 cpsps	GTS 40 -3 -2	1996
		cp4 cpsps	MON89788	2009
	Glufosinate	pat	A2704-12	2009

Source: [66]

V. DRAWBACK OF GENETIC ENGINEERING

[76] reported that genetically engineered crops/plants can have toxicity, allergenicity and genetic hazards and these arise from inserted gene and their expressed proteins, secondary or pleiotropic effects of the products of gene expression, and the possible disruption of natural genes in the manipulated organism. For instance, the starlik maize provides example of hazard food due to inserted gene using *Bacillus thuringinesis* for insect resistance, but unfortunately when people consume they were get allergic [77].

One big potential drawback of this technology is that some organisms in the ecosystem could be harmed, which in turn could lead to a lower level of biodiversity. When we remove a certain pest that is harmful to crops, we could also be removing a food source for a certain species. In addition, genetically modified crops could prove toxic to some organisms, which can lead to their reduced numbers or even extinction.

Cross-pollination can cover quite large distances, where new genes can be included in the offspring of organic, traditional plants or crops that are miles away. This can result in difficulty in distinguishing which crop fields are organic and which are not, posing a problem to the task of properly labeling non-GMO food products. Besides, genes from commercial crops that are resistant to herbicides may cross into the wild weed population, thus creating super-weeds that have become impossible to kill. For genetically enhanced vegetation and animals, they may become super-organisms that can out-compete natural plants and animals, driving them into extinction [76].

As previously mentioned, genetically modified foods can create new diseases. Considering that they are modified using viruses and bacteria, there is a fear that this will certainly happen. This threat to human health is a worrisome aspect that has received a great deal of debate. Studies found DNA from the M13 virus, Green fluorescent protein and Rubisco genes in the blood and tissue of animals [78]. In2012, a paper suggested that a specific micro RNA from rice could be found at very low quantities in human and animal serum [79].

VI. CONCLUSION

Genetic engineering plays important role in different aspects of human life such as in agricultural sectors, pharmaceutical, industrials and etc. Crop production has been facing different stresses which are categorized as biotic and abiotic. Though abiotic stresses are more seriously affecting production, biotic stresses has also an impact that need to be solved either through conventional breeding or modern technology of gene transfer (genetic engineering). The most important biotic stresses include insect, disease (bacteria, fungus and viruses), weed, nematodes and etc. Accordingly, genetic engineering will improve the resistance of our crops to these stresses through transferring gene or DNA without restriction to the species. However, this technology also has different demerits such as supper weed development, contamination of non-modified crops, and occurrence of new disease, allergenicity and disturbance of biodiversity. So, application of this technology needs deep understanding and proper management of modified crop to reduce its negative impact up on environment and social health.

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