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Genetic engineering for biotic stress

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Abstract
Resistance to biotic stress factors such as pests and diseases is the prime research topic across the world as it affects the yield of the crop significantly. Conventional plant breeding has been a successful technique to develop the biotic stress resistant cultivar but it is time taking and a laborious task. To speed up the breeding process and ensure site-specific breeding, scientists have been turning towards transgenic approaches to incorporate the desired gene into a plant variety. Many transgenic crops have been already commercialized and few are in the study phase. Bt cotton is one of the classical examples of a widely cultivated transgenic crop. Though transgenic crops are widely accepted by farmers across the globe, the argument of negative impact on health by the scientists is restricting the commercialization of crops. The advantages, disadvantages, and current status of transgenic breeding along with few successful studies are discussed in the current article.

Keywords: genetic engineering, crispr, virus mini-replicon, abiotic stress, rnai, gene silencing, cry genes

Introduction

In the present-day situation, food accessibility and quality are the major problems to be solved on priority, considering climate change. Climate change affects the yield of crops significantly and is also expected to reduce the availability of agricultural land in near future (Easterling *et al*. 2000). Crop yield reduction due to biotic stresses like pests, diseases, and weeds combined with the abiotic factors such as drought, salinity, and other environmental aspects pulls down the food production and agriculture economy in various parts of the world. Agriculture experts have already started working on various approaches to tackle food unavailability due to biotic factors. Strengthening of food production can be attained through various strategies such as increasing area under cultivation, improving agricultural practices, developing prominent varieties through traditional breeding, and following transgenic methods (Sree & Rajam, 2015) [40]. Traditional breeding has been successfully implementing in various parts of the globe to develop biotic stress-resistant varieties, but it is laborious and time-consuming (Mathews & Campbell, 2000) [27]. Therefore, modern genetic engineering approaches such as gene editing, CRISPR techniques, etc are being popularly used to develop the latest biotic stress-resistant varieties.

Plant variety improvement through transgenic breeding enabled the plant breeders, biotechnologists, and geneticists to develop the varieties with maximum accuracy and ease. Transgenic breeding for biotic stress involves analyzing the major biotic factors involved, its interaction with the plant gene, and transferring the gene to the desired plant genotype (Dunwell, 2000) [13]. After the launch of the first transgenic food crop, tomato, the area under cultivation of transgenic crops has been increasing gradually at a prominent rate (Dunwell, 2000) [13]. According to a study by the International Service for the Acquisition of Agri-biotech Applications (ISAAA) 191.7 million hectares of land were under transgenic crop cultivation in 2018. Although the cultivation of GMO crops has been increasing gradually, their effect on human health has been a major concern (Key *et al*., 2008) [22]. Tomato, soybean, cotton, maize, and canola are some of the important crops where breeding through transgenic strategies was fruitful. In the current article, the role of transgenic methods in the improvement of crops for abiotic stress has been discussed.

Biotic Stress Causing Factors

Crop species are under continuous natural biotic enemies, which reduce crop growth and yield.

Pathogen attacks such as viruses, fungi, bacteria, and nematodes play important role in knocking down the plant performance by causing an adverse effect on plants. Apart from this, competition from weeds for nutrients also causes a considerable yield loss across the world. All these stress factors accompany each other during the crop growth resulting in economic damage (Pandey *et al*., 2017) [34]. These biotic factors can be avoided by controlling agents and resistant varieties. Resistant varieties are the most economical option compared to controlling agents. Biotic stress-resistant varieties have been developing through the conventional breeding methods but being laborious and time taking, it is being overtaken by transgenic approaches.

Importance of Transgenic Plant Breeding for Biotic Stress Tolerance

Though the biotic stress controlling agents and conventional breeding approaches aided in controlling the biotic factors, the emergence of resistance breaking biotic races is rapid and very common (Network, 2016) [28]. Developing the plant genotypes at a pace equal to the newly evolving biotic stress factors is possible only through the transgenic techniques as the conventional breeding methods are time-consuming. The major advantages of transgenic breeding are problem-specific breeding, less

laborious, fast, economical, and accurate. It also has some drawbacks such as loss of biodiversity, adverse effects on human health, and risk of spreading the transgenes to wild plants in nature (Pusta *et al*., 2008) [35].

Several prominent research studies have been taken place in the last few years for the resistance of various biotic factors. Gene editing technologies were successfully utilized to develop resistance for bacteria, viruses (Wally & Punja, 2010) [44], nematodes (Atkinson *et al*., 2003) [4], and many other biotic stress-causing agents. The last few years had witnessed some site-specific genetic engineering events using zinc-finger nucleases (ZFNs), meganucleases, and clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated protein 9 (Cas9). All these methods can be implemented for biotic stress tolerance in plants (Borrelli *et al*., 2018) [6].

Genetic engineering for various biotic stresses

Virus

Viruses are the microbial particles that enter the plant through cut wounds and multiply inside the plant. Plant viruses cause greater damage to the plants which results in a significant yield reduction. It is proved that the virus causes the most problematic diseases in plants. (Anderson *et al*., 2004) [3]. Resistant plant varieties are the only solution to avoid the virus completely. Strong virus resistance genes are required to apply this strategy in plant improvement. The application of transgenic techniques for virus resistance has been in application since the 1980s (Savathri *et al*). The first application of transgenics for virus resistance was studied by Powell-Abel (Abel *et al*., 1986) [1]. His study depicted that tobacco plants with tobacco mosaic virus coat protein gene were resistant to Tobacco Mosaic Virus. Other examples of virus-derived genes include replicase (Gonsalves, 1998) [18], RNA, resistance protein (Foster *et al*., 2009) [15], etc.

RNA silencing is one of the latest genetic engineering techniques that have been implementing globally to develop virus-resistant cultivars. In the RNA silencing system fragments of sense and antisense viral nucleic acid sequences will be introduced into chromosome plants, which causes resistance to viral particles in plants. Virus mini-replicon (Brumin *et al*., 2009) [7] and expression of artificial microRNA (Niu *et al*., 2006) [30] are some of the other prominent methods used for virus resistance in plants. The development of transgenic papaya to control papaya ringspot virus in Hawai is one of the prominent milestones in the development of virus-resistant cultivars. SunUp and Rainbow are the two cultivars developed by using the coat protein gene and released commercially in 1998. It inculcated new hope and confidence in the papaya industry of Hawaii and rescued the people dependent on papaya cultivation and processing (Gonsalves, 1998) [18]. This depicts the success and importance of the transgenic strategies in the development of virus-resistant cultivars.

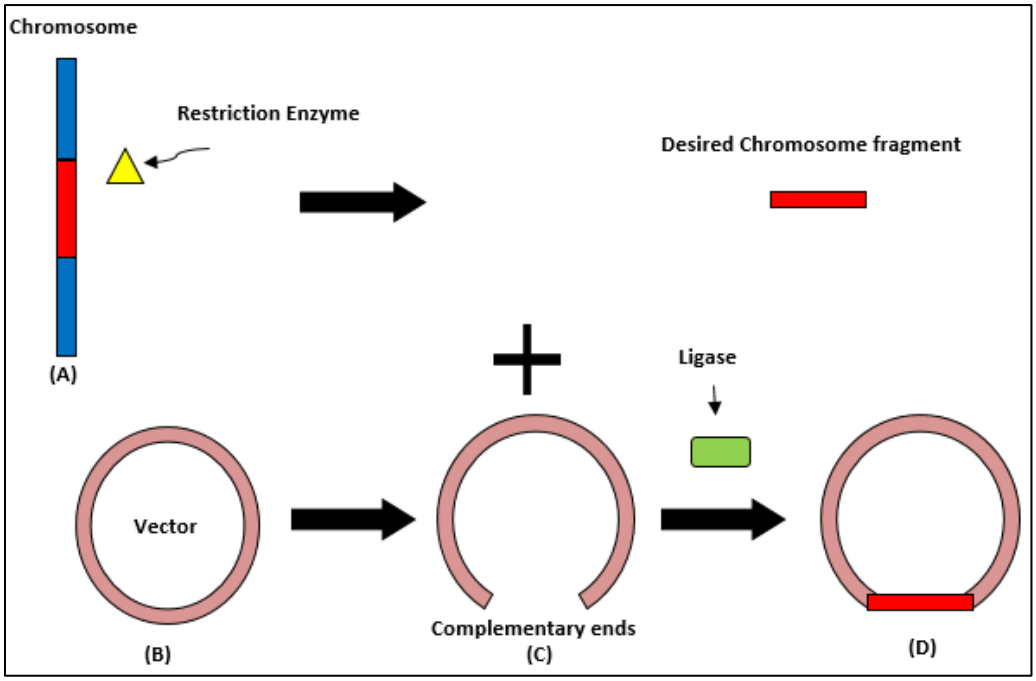

Fig 1: A general outlook of genetic engineering process.

(A) Cut the DNA fragment holding the desired gene using a restriction enzyme. (B) Select a vector and create complementary ends by using same restriction enzyme. (C) Mix the desired fragment with the vector and ligase. (D) The final transgenes outcome is then multiplied in the desired host and expressed.

Bacteria and Fungi

Bacteria and fungus cause a wide range of diseases in crops decreasing the yield drastically. Multiple agronomic practices as well as breeding practices have been following to avoid bacterial and fungal infection. Research studies over the last few years found several transgenic methods to avoid or reduce bacterial and fungal infection (Salomon & Sessa, 2012) [37]. The most important among those is the expression of resistance genes (R-gene). R-genes code for nucleotide-binding peptides harboring leucine-rich repeats (LRRs) which are transmembrane in nature. These R genes are being used in plant breeding frequently to developed disease-resistant cultivars (Saharan *et al*., 2016) [36].

These genes are dominant and offer resistance for one or multiple diseases. Transferring the R gene to the desired genotype by using transgenic methods has several additional advantages for disease control. They have a natural mode of action that is similar to the plant immune response system and no additional efforts are required from farmers. However, R genes sometimes lose their resistance in nature due to co-evolving genes (Wally & Punja, 2010) [44].

Fungal organisms usually produce toxins called mycotoxin which is responsible for the virulent nature of the fungus (Kimura *et al*., 2006) [24]. Keeping in view, how the fungus deals with the mycotoxin, the scientists developed transgenic lines of various crops such as wheat, rice, and barley. The transgenic wheat resulted in declined spike infection (Okubara *et al*., 2002) [32], transgenic rice(Ohsato *et al*., 2007), and barley (Manoharan *et al*., 2006) [26] depicted higher yield with less mycotoxin.

Advancement of genome editing technologies such as the CRISPR technique enabled plant scientists to develop disease-resistant cultivars with precise DNA modifications. Kim *et al*., successfully utilized CRISPER / Cas9 oriented mutagenesis of the Os8N3 gene in Kitaake rice cultivar. The edited Os8N3 revealed enhanced resistance to Xanthomonas bacteria in the homozygous mutant lines of the Kitaake rice cultivar (Kim *et al*., 2019) [23].

Table 1: Some prominent genetic engineering events occurred in various crops for disease resistance.

Crop	Gene Expressed	Target Pathogen	Year of Approval	References
Squash	Coat Protein	Zucchini Mosaic Virus	1994	(Tricoli *et al*., 1995)
Squash	Coat Protein	Watermelon mosaic virus	1996	(Tricoli *et al*., 1995)
Potato	Replicase and Helicase	Potato leafroll virus	1998	(Thomas *et al*., 1997),(Kaniewski & Thomas, 2004)
Papaya	Coat Protien	Papaya ring spot virus	1996	(Gonsalves, 1998) [18]
Sweet pepper	Coat protein	Cucumber mosaic virus	1998	(Zhu *et al*., 1996)
Potato	Coat protein	Potato virus Y	1999	(Newell *et al*., 1991), (Kaniewski & Thomas, 2004)
Tomato	Coat protein	Cucumber mosaic virus	1999	(Yang Rongchang *et al*., 1995)
Bean	RNA of viral replication protein	Bean golden mosaic virus	2011	(Bonfim *et al*., 2007)
Papaya	Coat protein	Papaya ringspot virus	2009	(Davis & Ying, 2004)

Insects

The success of gene editing for insect resistance started with the detection of *cry* genes from Bacillus Thurengensis bacteria (Vaeck *et al*., 1988) [43]. These genes code for the proteins called Bt toxins. These toxins prompt lytic pore formations in the insect gut causing the death of the insect on consumption. (Lightwood *et al*., 2000). Since the first tobacco and tomato cultivars with *cry* genes were developed tissue-specific endotoxins coding genes were injected into several crops against specific pests (Vaeck *et al*., 1988) [43].

VIP genes from Bacillus Thurengensis are also the genes that code for insect resistance. Their expressions are majorly confined to sporulation and detected from the starting of the vegetative phase and sporulation phase of bacteria (Estruch *et al*., 1996) [14]. These genes cause swelling and disruption of the insect's midgut epithelial cells and result in its death. So far, 50 VIP proteins were identified by scientists (Dar *et al*., 2017) [9]. Other than microorganisms, genes from the insect itself can be used to develop insect resistance. Chitinase is one such example of those genes expressed during the molting of the larva. Insects when fed on the plants with the chitinase gene get intimate to the chitinase enzyme and undergo death due to disturbance of their molting process. Research studies by Ding *et al*., (1998) [11] revealed that the plants expressing the chitinase gene have resistance to budworm and hornworm larvae.

The commercialization of Bt cotton in India is one of the prominent examples of the success of transgenic crops. Bt cotton is a genetically modified cotton cultivar that is resistant to bollworm attack. It was developed by incorporating the genes which encode toxin crystals of endotoxins (Siddiqui *et al*., 2019) [39]. Cotton cultivars with Vip genes were also developed to tackle the insect problem in the cotton crop. Even though the insecticidal effect of the cotton cultivars with Vip genes is high the license granted for these cultivars for commercial cultivation is low across the world (Christou *et al*., 2006) [8]. Today almost 90% of Indian cotton cultivation is based on Bt cotton. Such a wide acceptance of transgenic cotton reveals the importance and success of transgenic crops for disease resistance.

Table 2: Major genes utilized for insect pest resistance in genetic engineering studies

Crop	Resistance to	Gene Inserted	Year
Cotton	Bollworm	Cry gene Complex	1987
Brinjal	Fruit borer	Cry 1 Ab	2004
Cabbage	Cabbage worm and looper	Crygene Complex	2002
Maize	European corn borer	Cry gene Complex	1995
Okra	Shoot and fruit borer	Cry1 AB	2002
Potato	Colorado Potato beetle	Cry gene Complex	1995
Apple	Codling moth	Cry 1 Ac	2000
Soybean	Leaf eating caterpillar	Cry 1 Ac	1996
Sugarcane	Stem borer	Cry 1 Ab	1997
Tomato	Tobacco hornworm	Cry 1 Ac	1987
Tobacco	Leaf eating caterpillar	Trypsin inhibitor gene from cowpea	2001

Nematode Parasites

Nematode parasites cause significant yield losses in cereal and vegetable crops. Agronomic practices and controlling agents like fumigants are the old practices that have been following to reduce nematode infestation. Researchers have shown that Genetic engineering leading to gene transformation alters the plants into nematode-resistant (Ali *et al*., 2017) [2]. The genetic engineering approaches for nematode resistance include protease inhibitor gene cloning, anti-nematode protein application, resistance gene transfer, and application of RNA interference to crush nematode effectors.

Transferring nematode resistance genes through gene-editing techniques is the most popular method of genetic engineering for nematode resistance. *Hs1pro-1* from *Beta procumbens* for the resistance of beet cyst nematode *H. schachtii*, *Gpa*-2 from potato against the nematode *Globodera, and Hero A* from tomato against *G. rostochiensis* are some of the classical examples of gene transferring studies for nematode resistance (Fuller *et al*., 2008) [16]. Another prominent study involving nematode resistance was performed by Paal *et al*., 2004. They found the transfer of nematode resistance gene Gro1-4 induced the resistance towards the nematode pathotype G. rostochiensis.

RNA interference (RNAi) is one of the emerging technologies that have been utilizing to induce resistance for various pathogens. In this technique, nematodes absorb double-stranded RNA (ds RNA) or short interfering RNAs (siRNAs) from the plants holding these RNAs, which extract a systemic RNAi response in nematodes (Gheysen & Vanholme, 2007) [17]. Huang *et al*., 2006 used this technique to induce resistance in Arabidopsis. Applying this approach, genetic engineering was done to Arabidopsis plants to express dsRNA of one of the parasitism genes, 16D10. These gene-edited plants were found to be resistant to four species of root-knot nematodes.

Herbicide resistance

Weeds compete with the crop for nutrients and cause considerable economic damage. The application of herbicides is the most common practice followed throughout the world to eradicate weeds in the field. Therefore, cultivars with herbicide resistance are essential. Glyphosate is the most common herbicide applied across the globe, which inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. Expression of this enzyme in genome-edited petunia plants depicted resistance to glyphosate (Shah *et al*., 1986) [38]. After the discovery of the herbicide-resistant petunia plant, Glyphosate tolerant soybean was the first commercially cultivated herbicide-tolerant cultivar launched in 1996. Later, herbicide-tolerant cotton, maize, etc were also developed (James, 2014). Latest studies revealed that the transformation of two genes, glyphosate acetyltransferase gat and EPSPS G2-aroA genes induced effective glyphosate resistance in tobacco (Dun *et al*., 2014) [12].

Conclusion

The major concern regarding the application of genetic engineering for biotic tolerance is the stability of expression over the generations. Therefore, special attention should be given while applying the transgenic methods so that the transferred genes are expressed in the next generations. Many cultivars were developed and are under development using transgenic approaches but only a few were commercialized. Bt brinjal is an example of such a case in India. The country developed transgenic BT brinjal many years ago but it is still not commercialized due to opposition from social organizations and some scientific experts. The major factor that helps in popularizing GMO crops is proof of safety. The GMO crops when proved to be safe for consumption by humans and animals, encourages the farmers to cultivate GMO crops, and scientists to conduct research on transgenic crops.

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