Review Paper:

Enhancing insect resistance in rice through biotechnological techniques

Nayak Gyanisha* and Chaudhari Prabharani
Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur- 492012 (C.G.), INDIA
*gyanishanayak@gmail.com

Abstract

Plants are subjected to a variety of biotic and abiotic stresses throughout their entire life cycle. Like other biotic stress factors, insect pests have raised severe issues about yield losses, putting agricultural productivity at risk. Rice is a key source of nutrition for the world's growing population and insect infestations are particularly severe in rice which grow in warm, humid climates. Many phytophagous insects find rice plants to be an attractive and nutritious food source. Hundreds of insect species cause damage to rice to varying degrees, but only a few cause major damage regularly. Insect resistance capacity can be improved either by breeding or by biotechnology to reduce yield loss in rice.

However, scientists have turned to biotechnological techniques because of the long duration in traditional breeding and restricted availability of gene(s) of interest in the primary gene pool. The purpose of this review is to analyse the current state of biotechnological intervention for the resistance of a few important insects in rice.

Keywords: Biotechnology, genetc resistance, insects, rice.

Introduction

Rice (*Oryza sativa*) is one of the world's most important crops supplying food over half of the global population. It is the staple food of more than 70% of Indians and is crucial to their food security. It is predominantly grown in States like West Bengal, Uttar Pradesh, Punjab, Odisha, Chhattisgarh, Bihar, Andhra Pradesh and Telengana. Rice is grown on 43.79 million hectares in India, with a production of 116.42 million tonnes and a yield of 2659 kg/ha[1]. Crop losses are caused by a variety of biotic and abiotic stressors.

Among the biotic stresses, insect pests remain a significant threat to enhanced rice production. Hundreds of insect species inflict damage to rice to various extents, but only a few occur on a regular basis and cause significant damage. Insects feed on all parts of a rice plant at all stages of development, reducing production. One of the most important aspects of meeting the rising demand for rice is the development and execution of appropriate rice pest management measures[14]. Insect control can be divided into two categories: biological (predators, parasites, natural pesticides etc.) and chemical (chemical pesticides, insecticides etc.). The biological process is inexpensive and has no negative consequences for the environment.

The best way of insect control in crop plants is genetic resistance. It is the ability of some genotypes to produce higher yields of good quality than susceptible kinds under equal environmental conditions and at the same starting level of insect attack. Both breeding and biotechnological approaches can be used to enhance insect resistance.

Traditional plant breeding techniques are more reliable for developing resistance in plants. The procedure, however, takes a long duration to complete. To overcome this major problem, marker-assisted selection (MAS) offers a method by which selection for specific traits can be greatly accelerated. MAS is more successful with relatively simple traits and inherited in a Mendelian fashion. However, genomic selection in combination with the increase in the resolution markers and the decrease in the cost, will result in enhanced breeding strategies that make use of huge amounts of genomic data, paired with estimated breeding values assigned to markers to speed up breeding processes and increase the rate of gain. The genetic mapping of QTLs (Quantitative Trait Loci) has been ongoing for many years. These QTLs identify the chromosome region where the gene(s) affecting traits are most likely to exist within a statistical range.

However, in order to fully benefit from QTLs, it is required to identify the genes responsible for trait variation and to comprehend the molecular basis of QTLs. Apart from breeding methods, biotechnological techniques have a significant role in introduction of a new trait to the plant which does not occur naturally in the species (e.g. resistance to certain insects, diseases, herbicides, environmental conditions or improving the nutrient profile of the crop *etc*.).

Through improvements in biotechnology, horizontal resistance breeding, in which resistance is based on many genes, is becoming increasingly popular as genetically enhanced sustained pest resistance with fusion genes[58].

Advantages of biotechnology over breeding

Choosing biotechnology over conventional approaches has advantages[7]:

i. It provides access to non-rice genes that are otherwise unavailable to rice breeders.

ii. It allows purified rice genes into rice after modifications that give enhanced performance not attainable through recombination and mutation *in vivo*.

iii. It allows the addition of specific character to rice without the linkage drag and the requirement for backcrossing that accompany sexual hybridization.

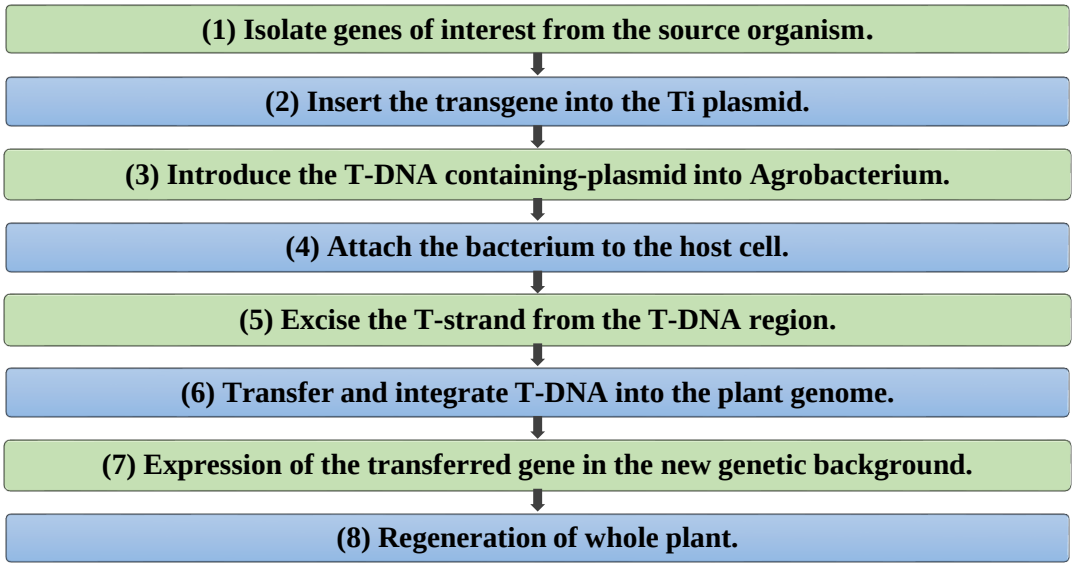
Biotechnological techniques

In biotechnology, a variety of approaches are employed for crop improvement. Some of them are utilised to create resistance against insect pests in rice.

***Agrobacterium*-mediated transformation:** From a commercial and biosafety standpoint, *Agrobacterium*-mediated transformation is acknowledged to be the ideal way of producing transgenic plants due to its great efficiency. *Agrobacterium tumefaciens* is a naturally occurring soil microbe known for infecting susceptible plant species with crown gall disease. *Agrobacterium tumefaciens*, which carries the tumor-inducing (Ti) plasmid, causes galls on the roots and crowns of many dicot angiosperm species as well as some gymnosperms, whereas *Agrobacterium rhizogenes*, which carries the root-inducing (Ri) plasmid, causes abnormal root production on the host plants.

The production of oncogenes is found in transferred-DNA (T-DNA) carried from these bacteria into the plant nucleus and integrated into the plant genome and the plant then reads and expresses the transferred genes as if they were its own. *Agrobacterium*-mediated gene transfer is known to result in the integration of foreign genes at a single locus in the host plant without the use of a vector backbone and to create marker-free plants which are required for transgenic crop commercialization.

The following are the steps in the Agrobacterium-mediated transformation process:



Protoplast fusion: Recent advancements in tissue-culture technology have created new possibilities for combining genes from various plant sources. Protoplast fusion is a technique in which cells in a culture media are stripped of their protective walls utilizing enzymes such as pectinase, cellulase and hemicellulase. These stripped cells, known as protoplasts, are collected from various sources and fused together using various ways such as electrical shock, PEG (Polyethylene Glycol) method and so on. When two protoplasts merge, a somatic hybrid is formed that contains genetic material from both plants.

Particle bombardment: Particle bombardment, also known as biolistics, is a common technique for genetically modifying plants and other organisms. The DNA to be used for transformation is coated on gold or tungsten particles (1–2 µm). The coated particles are put into a particle gun and accelerated to high speed utilizing electrostatic energy supplied by a droplet of water subjected to high voltage or compressed helium gas; the target might be plant cell suspensions, callus cultures, or tissues. Plant cell walls and membranes are penetrated by the bullets. Transgenes are liberated from the particle surface as the microprojectiles reach the cells and they are then incorporated into the plant's chromosomal DNA[41].

CRISPR/Cas9: CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) is a latest genome editing technology, which holds great promise because of its specificity, simplicity, efficiency and versatility by addressing key challenges posed by other genome editing tools[57]. It has two components: a CRISPR-associated endonuclease (Cas protein) and a guide RNA (gRNA/sgRNA). Cas9 is an enzyme that recognises and cleaves certain strands of DNA that are complementary to the CRISPR sequence using CRISPR sequences as a guide. The gRNA is a short synthetic RNA that contains a Cas-binding scaffold sequence as well as a user-defined approximately 20-nucleotide spacer that specifies the genomic target to be changed.

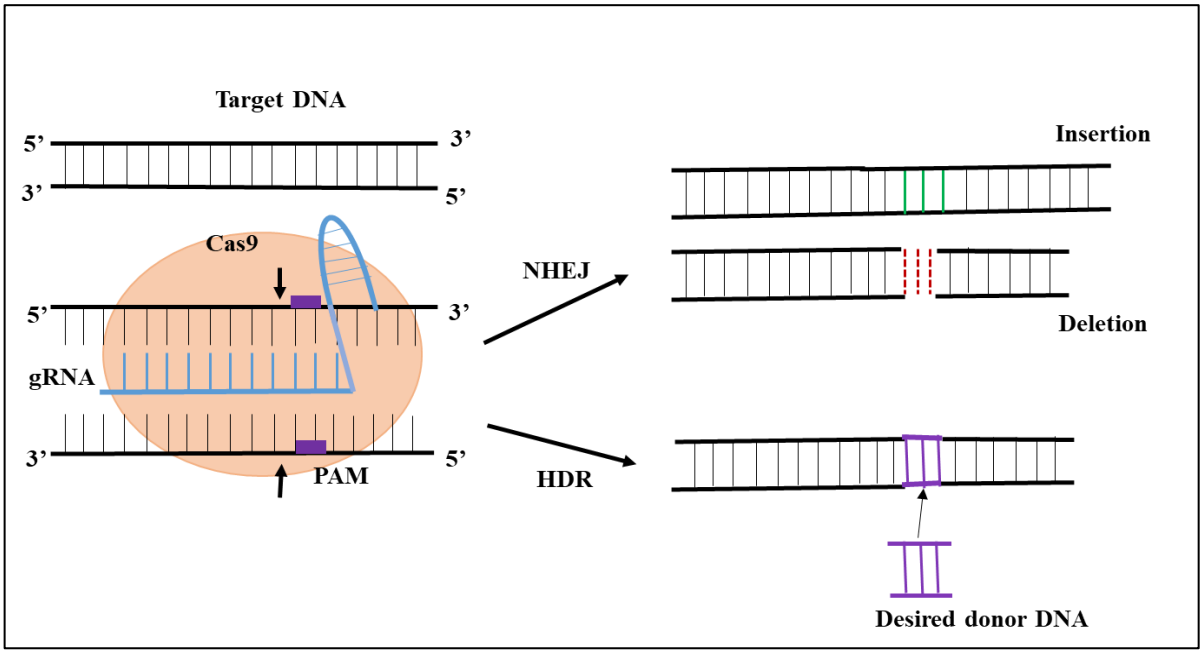


Fig. 1: Strategies of CRISPR/Cas9 based genome editing

The sgRNA binds to the Cas9 nuclease and instructs it to cleave complementary target DNA sequences close to a protospacer adjacent motif (PAM), usually the sequence NGG (where N represents any base), resulting in a double-strand break (DSB) in the DNA sequence[30].

Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are two key techniques for repairing Cas9 nuclease-induced DSBs[50]. NHEJ will result in insertion or deletion (Indel) mutations that disrupt the open reading frame of target genes, allowing us to achieve knockout (KO) of the target DNA sequence. HDR, on the other hand, can be used to introduce specific mutations or insert sequences of interest in accordance with the invading DNA template via homologous recombination, allowing us to achieve knock-in of a specific gene. By using this method, both plant and insect genomes can be modified for insect pest management[56].

Map based cloning: Forward genetics demands the cloning of sequences underlying a particular mutant trait. Map based cloning or positional cloning is a tedious forward genetic approach, hampering the quick identification of candidate genes. With the unprecedented advancement in whole genome sequencing and possibly even more so with the advent of saturating marker technologies, map-based cloning can now be done so quickly that candidate genes can now be identified in a couple of months, at least for some model plants. As a result, the use of map based cloning to isolate genes involved in natural variation and genes producing phenotypic mutations as determined by mutagenesis screens has increased nowadays[43].

Three major steps involved in map based cloning are:
(i) Isolation of a mutant strain with a recognizable change in phenotype and mapping of the mutant allele within a short genomic region to identify a pair of markers flanking the mutant allele;
(ii) Use of these markers for the identification and isolation of the DNA fragment containing the mutant allele and
(iii) Determination of the function of concerned gene.

Rice biotechnology and insect resistance

Brown Plant Hopper (*Nilaparvata lugens*): The brown plant hopper (BPH) is a rice insect pest that has been regarded as one of Asia's major rice production restrictions[32]. The insect pest is currently regarded as Asia's most devastating rice pest[33]. In susceptible cultivars, BPH infection can cause direct and indirect yield losses of 20–80 percent[3]. BPH attacks caused significant damage in China, Japan, Korea and Vietnam. Between 2005 and 2008, BPH attack caused yield losses of 2.7 million tonnes of rice in China alone. BPH-transmitted diseases like rice's ragged-stunt virus and grassy-stunt virus resulted in a decrease of 400,000 tonnes in Vietnam[12]. The most serious BPH outbreak in India happened in Kerala state between the end of 1973 and the beginning of 1974[40].

Molecular breeding promises a long-term solution for developing BPH resistant rice, however, the method's usefulness is limited by enormous genetic drag and time and resource needs as discussed earlier. As a result, using biotechnology to introduce genetic variants from other sources provides an alternate technique for managing BPH in rice. Under the control of a phloem-specific promoter from the rice sucrose synthase gene, as well as constitutively in the region of the maize ubiquitin promoter (*Ubi*), the snowdrop lectin gene (*Galanthus nivalis* agglutinin, GNA) was inserted into transgenic rice plants. According to insect bioassays and feeding observations, the expression of GNA in transgenic rice plants reduces the nymphal survival and overall fecundity of BPH[39].

Plant defensin is another protein class that confers resistance to fungus and bacteria and it has been thoroughly studied in plants[55]. BPH resistance is conferred by introducing the plant defensin *BrD1* from *Brassica rapa* into rice[19]. Although transgenic techniques to BPH resistance in rice have been successful, their deployment has been hampered by regulatory hurdles and political objections, notably in India and other developing countries.

The use of genome editing tools allows for the production of new alleles, the deletion of undesirable alleles/genes and the pyramiding of alleles without the need of linkage drags. CRISPR/Cas9 technology was used to modify the *CYP71A1* (encoding tryptamine 5-hydroxylase) gene in order to generate BPH resistant rice cultivars[36]. In rice, the modified plants displayed lower serotonin levels and higher salicylic acid (SA) levels, resulting in improved BPH resistance. CRISPR/Cas9 technology can be utilized to create novel allelic series from cloned BPH resistance genes, which could aid in the creation of broad-spectrum resistance in rice cultivars.

Yellow Stem Borer (*Scirpophaga incertulas)*: Because of its widespread distribution and chronic pattern of infection, the yellow stem borer (YSB) is the most devastating monophagous insect. It is the most destructive insect pest of rice in India, producing 3 to 95 percent production losses[51] and accounting for half of all insecticides used in rice fields[29]. YSB attacks the crop from seedling to harvest resulting in the complete loss of the affected tillers[49]. When insects attack during the vegetative stage, it causes dead heart and during the ear development stage causes white head[21]. Due to poor understanding of the genetics of resistance, lack of adequate germplasm and screening methodologies progress in developing rice cultivars resistant to YSB has been slow.

On the other hand, rice germplasm with a high level of resistance to the common YSB has been limited[9]. In the past, the lack of a high level of resistance to the YSB had slowed the creation of appropriate cultivars[8]. However, so far no gene has been specifically identified imparting tolerance to YSB[21].

The crystal insecticidal protein (δ-endotoxin) genes or cry gene proteins (Bt toxins) of *Bacillus thuringiensis* (Bt) are particularly poisonous to Lepidopterans[20], Dipterans[2] and Coleopteran insects[26]. The first transgenic IR62 rice containing the Bt gene was developed in India[42]. Since then, various organizations have used Bt genes like *Cry1Ab*, *Cry1Ac* and others to change rice types like IR64, Karnal Local and others to achieve resistance to YSB[31]. Several research groups have transformed rice with Bt genes and tested their efficiency against YSB in both greenhouse[18] and field conditions[4-6,13].

The majority of transgenic lines utilized in field studies expressed *Cry1Ab*, *cry1Ac*, *cry2A*, or a fused gene from *cry1Ab/cry1Ac*. *Agrobacterium*-mediated genetic transformation is reported to be resistant to Indica rice cultivars.

Table 1
Genes and QTLs identified for BPH resistance in rice[22]

Gene/QTL	Chromosome	Germplasm	Linked Markers
Bph22(t)	4	*O. rufipogon*	RM8212-RM261
Bph23(t)	8	*O. rufipogon*	RM2655-RM3572
Bph24(t)	—	IR73678-6-9-B (*O. rufipogon)*	—
bph25(t)	6	ADR52	S0010-RM8101
BPH26	12	ADR52	DS72B4-DS173B
BPH27	4	GX2183 (*O. rufipogon)*	RM16846-RM16853
Bph27(t)	4	Balamawee	Q52-Q20
Bph28(t)	11L	DV85	InDel55-InDel66
bph29	6	RBPH54 (*O. rufipogon)*	BYL8-BID2
bph30	10	RBPH54 (*O. rufipogon)*	RM222-RM244
BPH30	4	AC-1613	SSR28-SSR69
BPH31	3	CR2711–76	PA26-RM2334
BPH32	6	PTB33	RM19291-RM8072
BPH33	4	KOLAYAL/POLIYAL	H99-H101
BPH34	4	*Oryza nivara*	RM16994-RM17007
qBPH3	3	IR02W101 (*O.officinalis*)	t6-f3
qBPH4	4	IR02W101 (*O.officinalis)*	P17-xc4–27
qBPH4.2	4	IR65482–17-511 (*O. australiensis*)	RM261-XC4–27
qBPH4.3	4	Salkathi	RM551-RM335
qBPH4.4	4	Salkathi	RM335-RM5633

* Genes identified in last ten years

However, Ramesh et al[45] in 2004developed YSB-resistant transgenic indica rice lines with synthetic *cry1Ab* and *cry1Ac* genes as well as the snowdrop lectin gene GNA. This was the first study to use *A. tumefaciens* pSB111 super-binary vectors to successfully introduce three exotic resistant genes into diverse indica rice lines. *Agrobacterium*-mediated transformation was used to introduce a novel synthetic *cry2A* gene into the elite indica rice restorer line Minghuli 63[16].

Chen et al[17] tested 10 transgenic Bt rice lines generated from the same variety Minghui 63 against YSB and Asiatic rice borer using different Bt genes (five *Cry9C*, three *Cry2A* and two *Cry1Ac* lines). Toxicity to these two rice borers was substantial in all transgenic lines. Rice plants containing *cry1Ab* or *cry1Ac* have been obtained by using protoplast or particle bombardment methods[23].

Transgenic production of certain enzymes (chitinase, trypsins and other serine proteinases like chymotrypsin, elastases etc.) has been proposed as a possible alternative to Bt genes. *In vitro* experiments were used to measure trypsin activity in the midguts of YSB in order to find plant proteinase inhibitors that could be used in transgenic techniques to create insect resistant crops[38]. Bhutani et al[10] reported the development of transgenic Indica rice plants expressing potato proteinase inhibitor 2 (*Pin2*) genes with increased resistance to YSB. The cowpea trypsin inhibitor (*CpTi*) transgene has also been employed to develop stem borer resistance[11]. Yellow stem borer papain and midgut proteases were strongly inhibited by a protease inhibitor isolated from ripe jackfruit seeds, showing the potential utility of utilizing jackfruit protease inhibitor to protect rice plants from YSB damage[52].

Rice Leaffolder (*Cnaphalocrocis medinalis)*: Rice leaffolder was previously a minor pest that has now become a serious problem throughout the country, particularly in places where fertilizer use is excessive. *Cnaphalocrocis medinalis*, *Marasmia patnalis and Marasmia exigua* are three leaf folder species found in Eastern India with *Cnaphalocrocis medinalis* being the most frequent and widespread[46]. Depending on the agro-ecological environment, yield losses can range from 63 percent to 80 percent[44].

Transgenic rice lines, expressing *Cry1Ac* and Cowpea Trypsin Inhibitor (CpTI) showed resistance against *Cnaphalocrocis medinalis*. In the future, these lines could be employed as an alternate pest-control strategy and insect resistance management for Bt rice[25]. Kumar et al[34] tested 10 independently developed single-copy lines of Japonica (TNG67) and fragrant indica rice cultivars (HBC 19 and Pusa Basmati 1) for resistance to the rice leaffolder using third generation transgenic plants containing the potato proteinase inhibitor II gene (*pinII*). Five transgenic lines out of ten showed high levels of resistance to rice leaffolder with larval mortality ranging from 87.5 percent to 92.5 percent, demonstrating that *PinII* in rice can effectively regulate rice leaffolder.

Table 2
QTLs identified for rice leaf folder resistance[22]

QTLs	Chromosome	Germplasm	Linked markers
qRLF-1	1	Taichung Native 1	RM3412-RM6716
qRLF-2	2	Taichung Native 1	RM207-RM48
qRLF-3	3	Chuanjiang 06	RM1022-RM7
qRLF-4	4	Chuanjiang 06	RM3276-RM255
qRLF-8	8	Chuanjiang 06	RM72-RM331

Table 3
Genes identified for GLH resistance in rice[22]

Gene	Chromosome	Germplasm
GLH1	5	Pankhari 203
GLH2	11	ASD7
GLH3	6	IR8
glh4	3	PTB8
GLH5	8	ASD8, *O. rufipogon*
GLH6	5	TAPL796
GLH7	---	Maddani Karuppan
glh8	---	DV85
GLH9	---	IR28
glh10	---	IR36
GLH11	---	IR20965–11–3-3
GLH12	---	ARC10313
GLH13	---	Asmaita
GLH14	4	ARC11554

Manikandan et al[37] inserted a novel *cry2AX1* gene into the rice cultivar ASD16, which consists of a sequence of *cry2Aa* and *cry2Ac* genes driven by rice rbcS. Twenty of the 27 potential rice transformants tested positive for the *cry2AX1* gene. In insect bioassay, larval mortality ranged from 83.33 percent in T_0 transgenic rice plants to 83.33-90.00 percent in T_1 transgenic rice plants. This gene can be utilized to create transgenic rice plants that are resistant to the rice leaffolder.

Green Leafhopper (*Nephotettix virescens)*: The green leafhopper (GLH) is found all over Asia, however it is more prevalent in the tropics Sand subtropics. It can reduce yields by directly feeding or acting as a vector for the spread of tungro disease. GLH outbreaks were severe in India in 1968 and 1969 and in the Philippines in 1971. Previously, the peak population of green leaf hoppers was observed during the Diwali festival in the 1980s and 1990s, after which the population began to decline; however, since 2001, the GLH population has continued to rise beyond the Diwali[53].

Resistance against hopper pests such as BPH, GLH and WBPH (White Backed Plant Hopper) has been imparted due to a successful transformation of rice variety Chaitanya with the snowdrop (*Galanthus nivalis*) lectin gene, GNA[39]. The expression of GNA in rice has been shown by Tang et al[54] to reduce survival ability and fecundity as well as to delay the development of BPH. Additionally, the garlic lectin gene ASAL (mannose binding *Allium sativum* leaf agglutinin) has been proven to be an antifeedant and has insecticidal properties against BPH and GLH. It has been utilized to give hopper resistance in rice by transforming IR64[47].

The creation of a complex that occurs from the ASAL binding to the receptor molecule, an endosymbiotic chaperonin symbionin, located in the insect gut, has been shown to produce the insecticidal ability of ASAL against sap sucking insects. Similarly, the onion leaf lectin (*Allium Cepa* agglutinin, ACA) has been reported to have enough insecticidal properties to be a viable component in the transgenic method to sap sucking insect control management[28].

White Backed Plant Hopper (*Sogatella furcifera)*: White backed plant hopper (WBPH) attacks on paddy were first observed in India in 1903 in Surat, Pusa, Puna and Nagpur. Following that, it was seen in Bihar and Bengal in 1919, Jabalpur and its nearby Madhya Pradesh regions in 1960, Punjab in 1966 and Rajasthan in 1986. Andhra Pradesh, Assam, Bihar, Delhi, Haryana, Himachal Pradesh, Karnataka, Maharashtra, Manipur, Odisha, Tamil Nadu, Uttar Pradesh and West Bengal have also reported it[15]. In general, it is said to be more severe in places where resistant BPH cultivars have been planted. WBPH outbreaks have been reported in numerous Indian States.

The overexpression of the gene *OsWRKY89* led to increased leaf surface wax deposition, SA levels and lignification in culms, resulting in enhanced WBPH resistance[59].

Table 4
Genes identified for WBPH resistance in rice[22]

Genes	Chromosome	Germplasms	Linked Markers
WBPH1	7	Nagina 22	---
WBPH2	6	ARC10239	RZ667
WBPH3	–	ADR52	–
WBPH4	–	Podiwi A8	–
WBPH5	–	N'Diang Marie	–
WBPH6	11	Guiyigu	RM167
WBPH7	3	B5 (*O.officinalis)*	R1925-G1318
WBPH8	4	B5 (*O.officinalis)*	R288-S11182
WBPH9	6	Sinna Sivappu	RM589-RM539
WBPH10(t)	12	Sinna Sivappu	SSR12–17.2-RM28487
WBPH11(t)	4	Sinna Sivappu	Rm3643-rm1223
WBPH12(t)	4	Sinna Sivappu	RM16592-RM16649
Ovc	6	Asominori	R2373-C946
qOVA-1-3	1	Asominori	XNpb346-C112
qOVA-4	4	Asominori	R1854
qOVA-5-1	5	Asominori	XNpb251-R3313
qOVA-5-2	5	Asominori	C1268
qWPH2	2	*O. rufipogon*	RM1285-RM555
qWBPH5	5	*O. rufipogon*	RM3870-RZ70
qWBPH9	9	*O. rufipogon*	RG451-RM245
qWL6	6	Chunjiang 06	M3-M5
qWBPH3.2	3	IR54751	InDel3–23–InDel3–26
qWBPH11	11	IR54751	DJ53973-SNP56

Table 5
Genes identified for gall midge resistance in rice[22]

Genes	Chromosome	Germplasms	Markers
GM1	9	W1263	RM444-RM219
GM2	4	Phalguna	RM241-RM317
gm3	4	RP2068-18-3-5	RM17480-gm3SSR4
GM4	8	Abhaya	RM22551-RM22562
GM5	12	ARC5984	RM101-RM309
GM6	4	Duokang #1	RG214-RG476
GM7	4	RP2333–156-8	F8LB-SA598
GM8	8	Aganni	RM22685-RM22709
GM9	---	Line9	---
GM10	---	BG 380–2	---
GM11	12	CR57-MR1523	RM28574-RM28706

Map-based cloning and functional characterization showed that *BPH3* is actually a cluster of three genes encoding the plasma membrane-localized proteins, lectin receptor kinases (*OsLecRK1, OsLecRK2* and *OsLecRK3*). Plants that co-expressed all three genes had significantly improved BPH and WBPH resistance across the range[35]. *BPH6* expression facilitates exocytosis and cell wall reinforcement and induces coordinated SA, cytokinin (CK) and jasmonic acid (JA) signaling. This gene confers substantial resistance to all assessed WBPH and BPH biotypes without adversely affecting rice yields[24].

Gall Midge (*Orseolia oryzae*): Rice gall midge remained a widespread pest until the 1990s, when six biotypes emerged, inflicting significant losses in new areas such as Bihar and Manipur in the North East, as well as in certain traditional areas of Odisha, Andhra Pradesh, Madhya Pradesh and Kerala. It has not been a serious pest for about a decade, existing exclusively in endemic regions and producing minor yield losses[48]. Investigations further into the genetics of rice gall midge resistance at Raipur's Indira Gandhi Agricultural University (IGAU) led to the identification of ten gall midge resistance genes known as *Gm1* to *Gm10*. The discovery of the *Gm11* gene in the breeding line CR57-MR1523 brought the total number of known gall midge-resistant genes to 11[27]. Combining genes with multiple resistance mechanisms in a favorable agronomic background is recommended for long-term resistance.

Conclusion

Although we have made significant progress in the creation of insect resistant GM rice lines and are now entering the expansion phase of crop biotechnology, there are still certain areas where we can improve. There are several legitimate or imagined reservations about transgenics being the final solution to all pest problems.

The major limitations or concerns for transgenic plants are: stringent regulatory policies for risk assessment and lack of social acceptance for genetically modified crops worldwide, secondary pests are not controlled in the absence of sprays for the major pests. The need to control secondary pests through chemical sprays may kill natural enemies, thus offsetting one of the advantages of transgenics, high cost of producing and deploying transgenics, proximity to sprayed fields will reduce the benefits of transgenics and high cost of producing and deploying transgenics. As a result, more research regarding risk assessment, effective deployment and management of transgenic plants will be a key requirement for the long-term use of biotechnology for crop enhancement.

References

1. Agricultural Statistics at a Glance, Directorate of Economics and Statistics, Government of India **(2019)**

2. Andrews R.W., Fausr R., Wabiko M.H., Roymond K.C. and Bulla L.A., The biotechnology of *Bacillus thuringiensis*, *Crit. Rev. Biotechnol*., **6**, 163–232 **(1987)**

3. Balachiranjeevi C.H., Prahalada G.D., Mahender A., Jamaloddin M., Sevilla M.A.L., Marfori-Nazarea C.M., Vinarao R., Sushanto U., Baehaki S.E., Li Z.K. and Ali J., Identification of a novel locus, *BPH38(t)*, conferring resistance to brown planthopper (*Nilaparvata lugens* Stal.) using early backcross population in rice (*Oryza sativa* L.), *Euphytica*, **215**, 1–12 **(2019)**

4. Bashir K., Husnain T., Fatima T., Riaz N., Makhdoom R. and Riazuddin S., Novel indica basmati line (B-370) expressing two unrelated genes of *Bacillus thuringiensis* is highly resistant to two lepidopteran insects in the field, *Crop Prot*., **24(10)**, 870–9 **(2005)**

5. Bashir K., Husnain T., Fatima T., Zakia L., Mehdi S.A. and Riazuddin S., Field evaluation and risk assessment of transgenic indica basmati rice, *Mol. Breed.*, **13(4)**, 301–12 **(2004b)**

6. Bashir K., Husnain T. and Riazuddin S., Response of transgenic rice expressing two *Bt* genes to non-target insects, *Int. Rice Res. Not*., **29(2)**, 15–16 **(2004a)**

7. Bennett J., Cohen M.B., Katiyar S.K., Ghareyazie B. and Khush G.S., Enhancing insect resistance in rice through biotechnology. In: Carozzi N. and Koziel M. (eds), Advances in Insect Control: The Role of Transgenic Plants, Taylor and Francis Ltd., London, 75-93 **(1997)**

8. Bentur J.S., Host plant resistance to insects as a core of rice IPM, Science, Technology and Trade for peace and prosperity (IRRI, ICAR), McMillan India Ltd., 419–35 **(2006)**

9. Bhattacharya J., Mukherjee R., Banga A., Dandapat A., Mandal C.C. and Hossain M.A., A transgenic approach for developing insect resistant rice plant types. Science, Technology and Trade for peace and prosperity (IRRI, ICAR), Mc. Millan India Ltd., 245–64 **(2006)**

10. Bhutani S., Kumar R., Chauhan R., Singh R., Choudhury V.K. and Choudhury J.B., Development of transgenic indica rice plants containing potato proteinase inhibitor 2 gene with improved defense against yellow stem borer, *Phsiol. Mol. Biol. Plant*, **12(1)**, 43–52 **(2006)**

11. Brar D.S. and Khush G.S., Breeding rice for resistance to biotic stresses: conventional and molecular approaches, *Sabrao J.*, **45**, 225–34 **(2007)**

12. Brar D.S., Virk P.S., Jena K.K. and Khush G.S. Breeding for resistance to planthoppers in rice, In Planthopper, New Threats to the Sustainability of Intensive Rice Production Systems in Asia, International Rice Research Institute, Los Baños, CA, USA, 401–427 **(2010)**

13. Breitler J.C., Vassal J.M., Catala M.C., Meynard D., Marfa V. and Mele E., Bt rice harbouring cry genes controlled by a constitutive or wound inducible promoter: protection and transgene expression under Mediterranean field conditions, *Plant Biotech. J.*, **2(5)**, 417–30 **(2004)**

14. Chandllerr R.F., Management of Rice Insect Pests, Radcliffe's IPM World Textbook, https://ipmworld.umn.edu/heinrichs **(1979)**

15. Chelliah S. and Gunathilagaraj K., Rice pest management: Status and strategies. National Symposium on Rice in Wetland Ecosystem, Kottayam, Kerala, 54 **(1990)**

16. Chen H., Tang W., Xu C., Li X., Lin Y. and Zhang Q., Transgenic indica rice plants harboring a synthetic cry 2A gene of Bacillus thuringiensis exhibit enhanced resistance against lepidopteran rice pests, *Theor. Appl. Genet*., **111**, 1330–1337 **(2005)**

17. Chen H., Zhang G., Zhang Q. and Lin Y., Effect of transgenic *Bacillus thuringiensis* rice lines on mortality and feeding behavior of rice stem borers (Lepidoptera: Crambidae), *J. Econ. Entomol*., **101(1)**, 182–189 **(2008)**

18. Cheng X., Sardana R., Kaplan H. and Altosaar I., Agrobacterium transformed rice plants expressing synthetic *cry1Ab* and *cry1Ac* genes are highly toxic to striped stem borer and yellow stem borer, Proc. Natl. Acad. Sci., USA, **95(6)**, 2767–72 **(1998)**

19. Choi M.S., Kim Y.H., Park H.M., Seo B.Y., Jung J.K., Kim S.T. and Lee J.Y., Expression of *Br D*1, a plant defensin from *Brassica rap*a, confers resistance against brown planthopper (*Nilaparvata lugen*s) in transgenic rices, *Mol. and Cells*, **28**, 131–137 **(2009)**

20. Cohen B.M., Gould F. and Bentur J.C., Bt rice: practical steps to sustainable use, *Int. Rice Res*., **25(2)**, 4-10 **(2000)**

21. Deka S. and Barthakur S., Overview on current status of biotechnological interventions on yellow stem borer *Scirpophaga incertulas* (Lepidoptera: Crambidae) resistance in rice, *Biotechnol. Advan*., **28**, 70–81 **(2010)**

22. Du B., Chen R., Guo J. and He G., Current understanding of the genomic, genetic and molecular control of insect resistance in rice, *Mol. Breed*., **40**, 1-24 **(2020)**

23. Fujimoto H., Itoh K., Yamamoto M., Kayozuka J. and Shimamoto K., Insect resistant rice generated by a modified delta endotoxin genes of *Bacillus thuringiensis*, *Biotechnol*., **1**, 1151–5 **(1993)**

24. Guo J., Xu C., Wu D., Zhao Y., Qiu Y., Wang X., Ouyang Y., Cai B., Liu X., Jing S., Shangguan X., Wang H., Ma Y., Hu L., Wu Y., Shi S., Wang W., Zhu L., Xu X., Chen R., Feng Y., Du B. and He G., *Bph6* encodes an exocyst-localized protein and confers broad resistance to planthoppers in rice, *Nat Genet*., **50**, 297–306 **(2018)**

25. Han L., Wu K., Peng Y., Wang F. and Guo Y., Efficacy of transgenic rice expressing *Cry1Ac* and CpTI against the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée), *J. invertebrate Pathol*., **96**, 71-79 **(2007)**

26. Herrnstadt C., Soares George G., Wilcox Edward R. and David L., A new strain of *Bacillus thuringiensis* with activity against coleopteran insects, *Biotechn*., **4**, 305–308 **(1986)**

27. Himabindu K., Suneetha K., Sama V.S.A.K. and Bentur J.S., A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs, *Euphyt.*, **174**, 179–187 **(2010)**

28. Hossain M.A., Maiti M.K., Basu A., Sen S., Ghose A.K. and Sen S.K., Transgenic expression of onion leaf lectin gene in Indian mustard offers protection against aphid colonization, *Crop Sci*., **46**, 2022–2032 **(2006)**

29. Huesing J. and English L., The impact of Bt crops on the developing world, *Agbioforum*., **7(1)**, 84–95 **(2004)**

30. Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A. and Charpentier E., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, *Science*, **337**, 816–821 **(2012)**

31. Khanna H. and Raina S., Elite indica transgenic rice plants expressing modified *Cry1Ac* endotoxin of *Bacillus thuringiensis* show enhanced resistance to yellow stem borer, *Transgenic Res*., **11**, 411–423 **(2002)**

32. Korinsak S. et al, Improvement of submergence tolerance and the brown planthopper resistance of the Thai jasmine rice cultivar KDML105 by pyramiding *Sub1* and *Qbph12*, *Field Crop. Res*., **188**, 105–112 **(2016)**

33. Krishnaiah K. and Varma N., Changing insect pest scenario in the rice ecosystem—A National Perspective; Directorate of Rice Research, Rejendranagar, Hyderabad, India, 1–28 **(2011)**

34. Kumar R., Bhutani S., Singh R., Chauhan R., Chowdhury V. and Jain R., Enhanced resistance against the rice leaffolder (*Cnaphalocrocis medinalis*) in transgenic rice plants containing the

potato proteinase inhibitor II gene, *Entomol. Genera*, **32**, 11-22 **(2009)**

35. Liu Y., Wu H., Chen H., Liu Y., He J., Kang H., Sun Z., Pan G., Wang Q., Hu J., Zhou F., Zhou K., Zheng X., Ren Y., Chen L., Wang Y., Zhao Z., Lin Q., Wu F., Zhang X., Guo X., Cheng X., Jiang L., Wu C., Wang H. and Wan J., A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice, *Nat Biotechol*., **33**, 301–305 **(2015)**

36. Lu H.P., Luo T., Fu H.W., Wang L., Tan Y.Y., Huang J.Z., Wang Q., Ye G.Y., Gatehouse A.M.R., Lou Y.G. and Shu Q.Y., Resistance of rice to insect pests mediated by suppression of serotonin biosynthesis, *Nature Plants*, **4**, 338–344 **(2018)**

37. Manikandan R., Balakrishnan N., Sudhakar D. and Udayasuriyan V., Development of leaffolder resistant transgenic rice expressing *cry2AX1* gene driven by green tissue-specific rbcS promoter, *World J. Microbiol. Biotechnol*., **32**, 37 **(2016)**

38. Mazumdar S., Molecular characterization of trypsin genes in two economically important insect pests (*Scirpophaga incertulas* and *Helicoverpa armigera*) and recombinant soybean Kunitz trypsin inhibitor gene expressed in *Escherichia coli*, Delhi University, India, Ph.D. Thesis **(1996)**

39. Nagadhara D., Ramesh S., Pasalu I.C., Rao Y.K., Krishnaiah N.V., Sarma N.P., Bown D.P., Gatehouse J.A., Reddy V.D. and Rao K.V., Transgenic indica rice resistant to sap-sucking insects, *Plant Biotech. J.*, **1(3)**, 231–240 **(2003)**

40. Nalinakumari T. and Mammen K.V., Biology of the brown planthopper. *Nilaparvata lugens* (Stål.) (Delphacidae, Hemiptera), *Agric. Res. J*., **13(1)**, 53–54 **(1975)**

41. Narusaka Y., Narusaka M., Yamasaki S. and Iwabuchi M., Methods to transfer foreign genes to plants, transgenic plants-advances and limitations, *Intech Open*, DOI: 10.5772/32773 **(2012)**

42. Nayak P., Basu D., Das S., Basu A., Ghosh D., Ramakrishnan N.A., Ghosh M. and Sen S.K., Transgenic elite indica rice plants expressing *CryIAc* delta-endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*), Proc. Natl. Acad. Sci., USA, **94(6)**, 2111-2116 **(1997)**

43. Peters J.L., Cnudde F. and Gerats T., Forward genetics and map-based cloning approaches, *Trends in Plant Science*, **8(10)**, 484-491 **(2003)**

44. Rajendran R., Rajendran S. and Sandra P.C., Varietals resistance of rice of leaf folder, *Intl. Rice Res. News*, **11**, 17 **(1986)**

45. Ramesh S., Nagadhara D., Reddy V. and Ra K., Production of transgenic indica rice resistant to yellow stem borer and sap sucking insects, using super binary vectors of *Agrobacterium tumfaciens*, *Plant Sci*., **166**, 1077–1085 **(2004)**

46. Rath P.C., Bose L.K., Subudhi H.N., Lenka S. and Jambhulkar N.N., Biodiversity of insect pests of rice in India, *Internat. J. of Chem. Studies*, **8(1)**, 2998-3002 **(2019)**

47. Saha P., Majumder P., Dutta I., Ray T., Roy S.C. and Das S., Transgenic rice expressing *Allium sativum* leaf lectin with enhanced resistance against sap-sucking insect pests, *Planta*, **23**, 1329–43 **(2006)**

48. Sain M. and Prakash A., Changing pest scenario of cereal crops, National Conference on Pest management strategies for food security, IGKV, Raipur, 27-29 **(2008)**

49. Salim M. and Masir R., Efficacy of insecticides against rice stem borer at NARC, Islamabad, *Pak. J. Agri. Res*., **8**, 477–479 **(1987)**

50. Sander J.D. and Joung J.K.. CRISPR-Cas systems for editing, regulating and targeting genomes, *Nat. Biotechnol*. **32**, 347–355 **(2014)**

51. Senapati B. and Panda S.K., Rice stem borers in insect pests of cereals and their management, In Prakash Anand and Rao Jagadiswari, eds., Pub: AZRA, CRRI, Cuttack, Orissa, 3-18 **(1999)**

52. Shamim M., Khan N.A. and Singh K.N., Inhibition of midgut protease of yellow stem borer (*Scirpophaga incertulas*) by cysteine protease-like inhibitor from mature jackfruit (*Artocarpus heterophyllus*) seed, *Acta Physiol. Plant*, **33**, 2249–2257 **(2011)**

53. Singh S.K., Severe incidence of rice tungro disease in dry season, 2007- A case study, National conference on pest bio-diversity in rice and their management under changed climate, AZRA, India and CRRI, Cuttack, 15-16 **(2009)**

54. Tang K., Zhao E., Sun X., Wan B., Qi H. and Lu X., Production of transgenic rice homozygous lines with enhanced resistance to the rice brown plant hopper, *Acta Biotechnol*., **21**, 117–128 **(2001)**

55. Terras F.R., Eggermont K., Kovaleva V., Raikhel N.V., Osborn R.W., Kester A., Rees S.B., Torrekens S., Van Leuven F. and Vanderleyden J., Small cysteine-rich antifungal proteins from radish: Their role in host defense, *The Plant Cell*, **7(5)**, 573–588 **(1995)**

56. Tyagi S., Kesiraju K., Saakre M., Rathinam M., Raman V., Pattanayak D. and Sreevathsa R., Genome editing for resistance to insect pests: an emerging tool for crop improvement, *ACS Omega*., **5**, 20674–20683 **(2020)**

57. Vats S., Kumawat S., Kumar V., Patil G.B., Joshi T., Sonah H., Sharma T.R. and Deshmukh R., Genome Editing in Plants: Exploration of Technological Advancements and Challenges, *Cells*, **8(11)**, 1386 **(2019)**

58. Wan J.M., Perspectives of molecular design breeding in crops, *Acta Agron. Sin*., **32(3)**, 455–462 **(2006)**

59. Wang H., Hao J., Chen X., Hao Z., Wang X., Lou Y., Peng Y. and Guo Z., Overexpression of rice *WRKY89* enhances ultraviolet B tolerance and disease resistance in rice plants, *Plant Mol. Biol*., **65**, 799–815 **(2007)**.

(Received 19th October 2021, accepted 20th December 2021)