

Chapter 7

Biotechnological Interventions for Creating Novel Resistance Against Major Insect Pests of Rice



Pavneet Kaur, Kumari Neelam, Ankita Babbar, and Yogesh Vikal

7.1 Introduction

Rice is considered one of the most important cereal crops in the Asia-Pacific region. It has been estimated that half the world's population subsists wholly or partially on rice. Rice is mainly grown in tropical and subtropical areas worldwide spanning north at 53° N latitude and toward south at 39° S latitude and from sea level to altitudes of 3000 m. The warm and humid environment in which rice is grown is conducive to the proliferation of insects and pests. Globally, there are around 100 insect species to which rice plant remains vulnerable from sowing till harvest. The attack of insect pests is one of the major yield-limiting factors in rice causing up to 20–30% yield losses annually (Salim et al. 2001). Insects are the most abundant life form on earth, and their continuous evolution has become a major constraint to the global production of food and fiber. Insect pests, as a part of the natural ecosystem, pose serious constraints to the world's agricultural produce and thereby hamper the food security levels. Currently, many of the crops are suffering a yearly loss of about 36 billion USD in India due to insect pests (Dhaliwal et al. 2015; Rathee and Dalal 2018). In addition to direct impacts on yield, insects also reduce yields by making crops more susceptible to disease-causing pathogens (Haq et al. 2004). The insects/pests hamper the crop by negatively targeting the physiological and metabolic pathways at the different growth phases of rice. Several insects attack during the nursery stage leading to thrips (*Stenchaetothrips uniformis*), green leafhopper (*Nephotettix malayanus* and *N. virescens*), rice caseworm (*Nymphula depunctalis*), paddy stem borer (*Scirpophaga incertulas*), and swarming caterpillar (*Spodoptera mauritia*). In rice, a different range of biotic stress develops as a result of the infestation of insects

P. Kaur · K. Neelam (✉) · A. Babbar · Y. Vikal
School of Agricultural Biotechnology, Punjab Agricultural University,
Ludhiana, Punjab, India
e-mail: kneelam@pau.edu; yvikal-soab@pau.edu

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A. S. Tanda (ed.), *Molecular Advances in Insect Resistance of Field Crops*,
https://doi.org/10.1007/978-3-030-92152-1_7

in major field conditions, including stem borer (*Sesamia inferens*, *Scirpophaga incertulas*, *S. innotata*, *Chilo suppressalis*, *C. polychrysus*, *C. auricilius*), gall midge (*Orseolia oryzae*), swarming caterpillar (*Spodoptera mauritia*), leaf folder (*Cnaphalocrocis medinalis*), rice horned caterpillar (*Melanitis leda ismene* Cramer and *Mycalesis* sp.), yellow hairy caterpillar (*Psalis pennatula*), grasshopper (*Hieroglyphus banian*), rice hispa (*Dicladispa armigera*), whorl maggot (*Hydrellia philippina* Ferino), green leafhopper (*Nephotettix nigropictus*, *N. malayanus*, and *N. virescens*), brown planthopper (*Nilaparvata lugens*), white-backed planthopper (*Sogatella furcifera*), mealy bug (*Brevinnia rehi*), rice earhead bug (*Leptocorisa acuta*), and thrips (*Stenchaetothrips biformis*) (Plate 7.1).

The infestation of various insects follows different modes of action in order to infect the host plant. Majority of the insects are classified as chewing insects, piercing insects, and sucking insects. Chewing damage is caused by insects with mouthparts that lead to mechanical damage of tissues, thereby promoting ingestion. The latter type includes hoppers, responsible for invading plant cells and sucking nutrients from vascular tissues. However, the extend of disease occurrence is highly dependent on the severity and exposure frequency of insects.

Over the years, the widespread use of insecticides/pesticides has led to the evolution of pesticide-resistant insects and reduction in beneficial insect population, along with the harmful impact on food safety, humans, and the environment (Fitt 1994; Gatehouse et al. 1994; Gunning et al. 1991; Haq et al. 2004). These problems have led researchers to develop different insect control approaches using various tools and techniques of genetic engineering, molecular biology, and plant biotechnology that are more environmentally friendly. The various techniques used in terms of biotechnological aspects have been successfully devised in various crops for crop improvement, viz., attaining herbicide tolerance in soybean, cotton, corn, and canola crops (Gianessi 2005). Herbicide tolerance has been proven to be beneficial for farmers by increasing crop productivity and environmental benefits for soil and water quality and eliminating the need for manual removal of weeds. The current biotechnological approaches significantly aim for improving abiotic and biotic stress tolerance in various crops worldwide. Similarly, plant biotechnology targets a varied number of regulatory components associated with the growth and development of crops aiding in their evolution and domestication, by improving their respective quality and yield attributes. Another aspect of biotechnology involves genomic hybrid breeding, providing a promising approach for attaining true superior hybrids with the minimum cost expense (Plate 7.2).

Considerable progress has been made in the past to incorporate resistance against insects/pests of rice. All these methodologies exploit the prevailing phenomenon of host plant resistance in an environmentally favorable manner. The significant insect-pest damage in the case of economically valuable crops, like cotton, tobacco, tomato, corn, sorghum, sunflower, pulses, rice, maize, and wheat, can be reduced by employing the modern biotechnological tools through critical analysis and engineering of biological processes. In the insect research field, biotechnological tools have been applied to study various issues, such as insect identification, insect

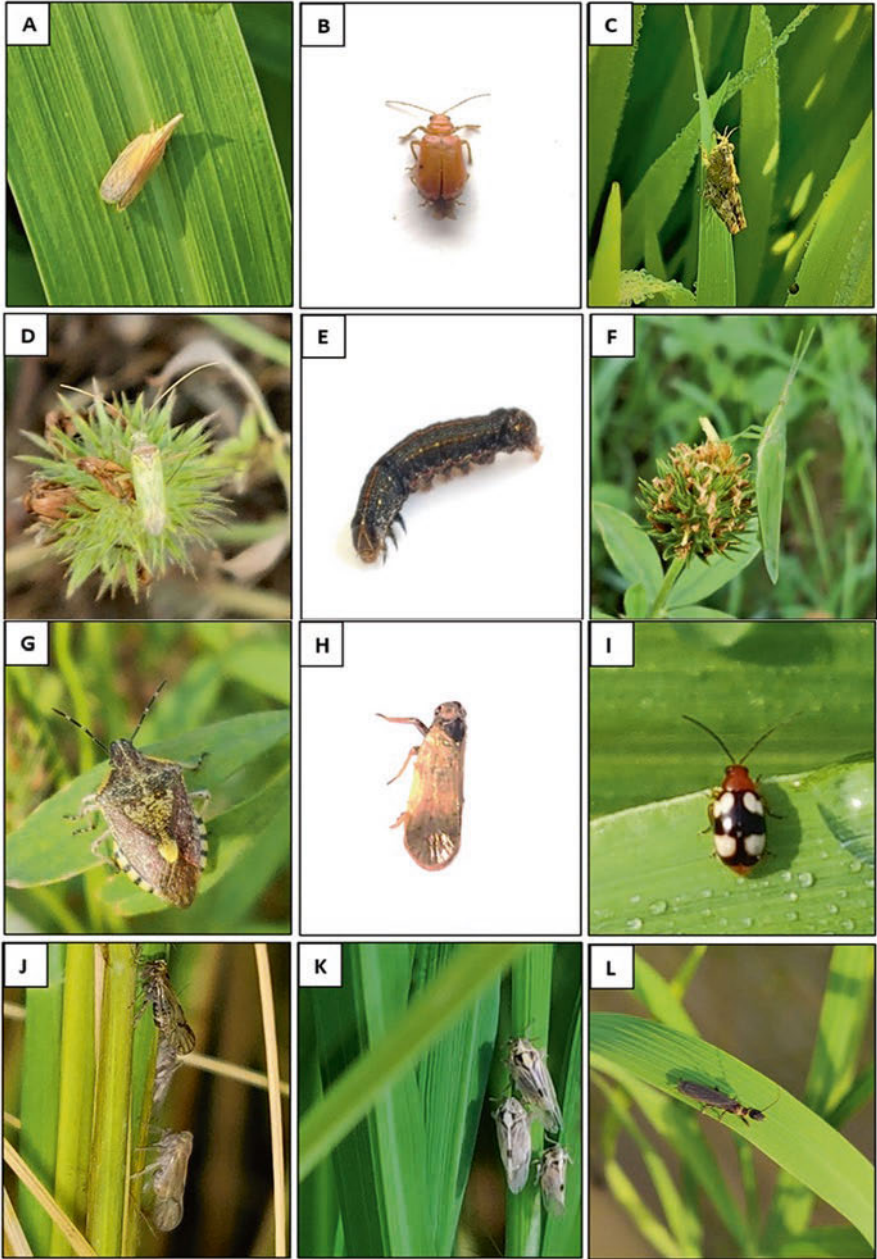


Plate 7.1 (a) Leaf hopper. (b) Stem borer. (c) Pygmy grasshopper. (d) Chinch bug. (e) Armyworm. (f) Chinese grasshopper. (g) Stink bug. (h) Rice delphacid. (i) Rice hispa. (j) Brown planthopper. (k) White-backed planthopper. (l) Rice thrip

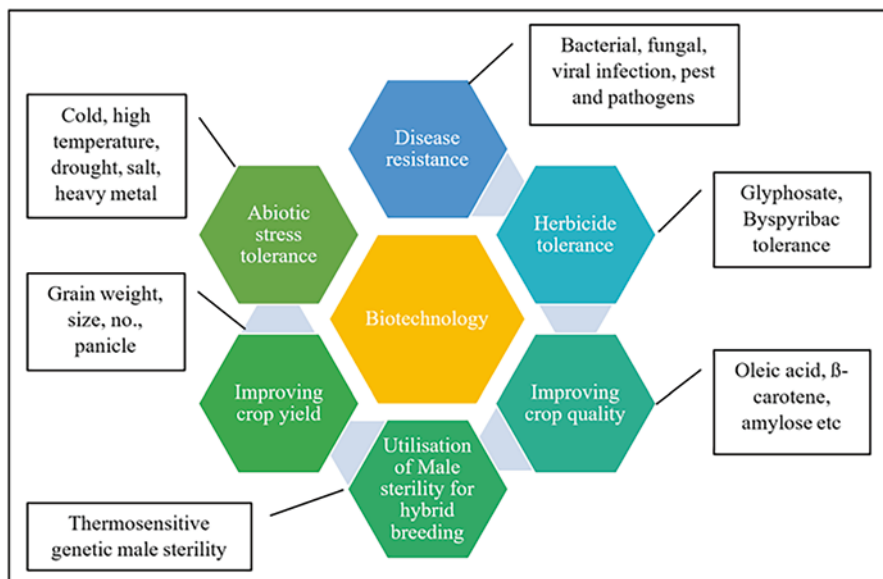


Plate 7.2 Applications of biotechnology in different aspects of crop improvement

control, and insect genetic relationships. It has a significant role in improving the potency and cost-effectiveness and in expanding the markets for bioinsecticides (Talukdar 2013). Genetic modification of the crops through biotechnology can potentially provide a much larger array of novel insecticidal genes along with conventional breeding. Since the commercialization of genetically modified crops in 1996, farmers have adopted the technology at such a dramatic rate, that in 2011, 16.7 million farmers in 29 counties planted 160 million hectares of biotech crops. In India alone, Bt cotton has increased cotton yields by up to 60% and has reduced insecticide sprays by around half. This in turn has led to an income increase of up to the US \$11.9 billion per annum (James 2011). Thus, the insect control strategies that integrate advanced knowledge in biotechnology will contribute to the sustainability of agriculture. Extensive knowledge regarding the genotype of insect-resistant rice using biotechnological approaches unveils a wide range of molecular mechanisms that can open new avenues in the field of improvement.

Crop protection through effective management of insect pests and pathogens has remained the primary target for various advances in biotechnology. These advances could take place by progressing in genetic engineering and molecular biology, which have resulted in identification, isolation, characterization, and modification of resistance genes from diverse biological sources. Employment of DNA-based markers provides additional efficiency and precision via marker-assisted selection for the introgression of various resistant genes in rice cultivars. Recombinant DNA

(rDNA) technology has significantly expanded conventional crop protection by providing dramatic improvement in manipulating genes from diverse and exotic sources and inserting them into microorganisms and crop plants to confer resistance to insect pests and increased effectiveness of biocontrol agents. The availability of fully characterized genes, in turn, led to the development of plant biotechnology, making the transgenic expression of such genes possible in crop plants. Several such genes have already been exploited in different crop plants irrespective of any genetic barrier. However, only a limited number of such genes have afforded desired field resistance to transgenic plants against limited insect pest species. Currently, biotechnology is being applied for the precise characterization of insect pest species as well as the identification and characterization of novel genes for meaningful insect resistance. RNA interference (RNAi), on the other, hand has emerged as a powerful technique for downregulating gene expression in insects, whereas CRISPR Cas involves genome editing techniques for understanding the functions of target genes in diverse organisms. Additionally, a systematic study of the complete repertoire of metabolites/chemicals of any organism has given birth to a new area of research called “metabolomics.” Integration of genomics and proteomics with metabolomics will enrich our understanding of the gene-function relationship that can be utilized in achieving crop improvement with a view to insect resistance. In this chapter, we will discuss various insect pests of rice, along with the biotechnological interventions, viz., genetic engineering, genomics, and the functional genomics approaches for managing the yield losses of rice.

7.2 Insects of Rice

The suitable environment favoring rice production promotes the proliferation of insects hampering its growth. These insects are enemies of rice production responsible for the reduction in total rice produce. The crop is attacked by more than 100 insect species, infesting varied plant parts by its specialized infesting organs and toxins (Table 7.1). Diverse insects attack the rice crop at a different stage of the life cycle. Majority of insects infesting rice plants attack during the vegetative stage belonging to the order Hemiptera, Homoptera, Orthoptera, Thysanoptera, Coleoptera, Lepidoptera, and Diptera. Among the insects attacking during the reproductive stage, green-horned caterpillar belongs to the minor pests of rice as its severity is too low. Among all insects, planthoppers, leafhopper, and leaf folders account for the cause of major alarming threats to rice production. Timely identification of insects is a key for accurate disease management strategy. The morphological identification of all these insects is aided by DNA barcoding differentiating insects in distinct species.

Table 7.1 Various insect-pests attacking rice crop at different developmental stages

Stage	Order	Family	Insect	Damage	Symptoms
Vegetative stage	Hemiptera	Pentatomidae	Black bugs (<i>Macropes excavatus</i>)	Phloem sap feeder	Stunting, wilting, chlorotic lesions, fewer tillers, panicles affected, and unfilled spikelet
	Homoptera	Pseudococcidae	Mealybugs (<i>Brevinnia rehi</i>)	Infest leaf sheath and act by sucking sap	Yellowing, stunting, small leaves, irregular tillering, and underdeveloped panicles
Orthoptera	Acrididae	Grasshoppers (<i>Hieroglyphus bantian</i>)	Cutoff leaf areas and panicles	Excessive foliage damage	
		Field crickets (<i>Gryllus pennsylvanicus</i>)	Feeds on seeds, tillers and roots	Patches appear, cutting off of tillers at ground level	
Thysanoptera	Thripidae	Rice thrips (<i>Stenchaetothrips biformis</i>)	Tearing of the leaf tissues	Silvery streaks, wilting, stunting, curling, and discoloration of leaves and seedling death	
Coleoptera	Chrysomelidae	Rice hispa (<i>Dicladyspa armigera</i>)	Adults target the upper surface of the leaf blade	White streaks and patches, withering of infected parts, reduced leaf area, less vigorous, stunted	
		Rice leaf folder (<i>Cnaphalocrocis medinalis</i>)	Larvae fold the leaves in the longitudinal direction with threadlike silk stitches	White stripe damage of leaf and vigor and photosynthetic ability hampered	
Lepidoptera	Noctuidae	Rice caseworms (<i>Paraponyx stagnalis</i>)	Larvae responsible for scrapping chlorophyll from leaves	Cutting of leaf tips and visible as cylindrical tubes	
		Rice green semiloopers (<i>Naranga diffusa</i>)	Young and mature larvae scrape tissue from leaf blades and feed on leaf edges	Scraped leaves exposing lower epidermis and damaged leaf edges	
		Armyworms (<i>Spodoptera frugiperda</i>) and cutworms (<i>Agrotis ipsilon</i>)	Larval feeding results in skeletonizing leaf blades	Leaves and panicle detachment	
		Pyrilidae and Noctuidae	Rice stem borers (<i>Scirpophaga innotata</i>)	Larval boring and feeding in the leaf sheath	Discolored areas, apical central leaf whorl browning and drying leading to affected tillers (dead heart), whitish empty panicles (whiteheads)

Reproductive stage	Diptera	Diopsidae	Stalked-eyed flies (<i>Teleopsis dalmani</i>)	Maggots feed on the growing zone of plant	Central whorl does not open, dries, and dies (dead heart)
		Cecidomyiidae	Rice gall midge (<i>Orseolia oryzae</i>)	Infestation of plants in seedbed only	Tillers appear in the form of tubular galls
Reproductive stage	Lepidoptera	Ephydriidae	Rice whorl maggots (<i>Hydrellia sasakii</i>)	Feeds on inner margins of unfurled leaves	Discolored areas, dried, drooped, wilted and deformed leaves, stunting, less tillering, delayed panicle initiation and maturity
		Satyridae	Green horned caterpillars (<i>Melanitis leda ismene</i>)	Larvae feed on leaf margins and blades	Removal of leaf tissue and veins
		Hesperiidae	Rice skippers (<i>Parnara mathias</i>)	Leaf tissue removal followed by leaf rolling	Removal of leaf tissue and veins and rolling of two edges of leaf tied with silken threads
		Delphacidae	Planthoppers:	Sap-sucking insects targeting xylem and phloem; transmits various viruses	Complete drying of plant, heavy infestation responsible for hopperburn (complete drying out)
			Brown planthopper (<i>Nilaparvata lugens</i>) White-backed planthopper (<i>Sogatella furcifera</i>) Small brown planthopper (<i>Laodelphax striatellus</i>)		
Cicadellidae	Leafhoppers (<i>Nephotettix nigropictus</i>)				
Ripening stage	Hemiptera	Pentatomidae	Stink bugs (<i>Halyomorpha halys</i>)	Feeds on endosperm of developing grains	Brown spots, shriveled and empty grains, discoloration of grains

7.3 Biotechnological Approaches

With the advent of genetic engineering and several tools of biotechnology, viz., genetic engineering tissue culture (anther culture, embryo culture), genetic transformation for insect resistance, inhibitors of several digestive enzymes, marker-assisted selection (MAS) for plant resistance to insect, pyramiding of resistant genes into a single cultivar, and development of insect-resistant plants using RNAi and CRISPR Cas have been accelerated. The acceptability of biotechnology products may be greater along with the increase in better understanding of biotechnological processes.

7.3.1 Genetic Engineering

The expanding knowledge regarding the genome and harboring genes has prompted advancement in the development of transgenics for the incorporation of resistance-conferring genes in commercially important rice varieties. Tissue culture offers the potential to contribute to the improvement of crop plants through the manipulation of plants at the cellular level. With the commencement of genetic transformation, it has become possible to replicate and introduce genes into the crop plants to produce resistance to insect pests. Insect-resistant genetically modified crops are offering great benefits for farmers. Gene resistance against various insects has been introduced into crop plants, such as maize, cotton, potato, tobacco, potatoes, rice, broccoli, lettuce, walnuts, apples, alfalfa, and soybean (Griffiths 1998). As the products of most transgenes are ingested by the insect pest and therefore act through the gut, most of the focus has been on transgene-encoded proteins that target the insect mid-gut and/or the peritrophic membrane to disrupt digestion or nutrition (Czapla and Lang 1990; Hopkins and Harper 2001; Murdock et al. 1990; Eisemann et al. 1994; Harper et al. 1998). Generally, the detrimental effects on larval and insect growth result from limited assimilation of nutrients (Williams 1999; Lopes et al. 2004; Zavala and Baldwin 2004; Silva et al. 2006). The use of transgenic plants that express insecticidal agents thus reduces the population of insect pests, usage of chemical insecticide, and the ecological damage they may cause (Schuler et al. 1998). To date, the most successful transgenes for insect control have been the genes encoding insecticidal toxins from the soil bacterium *Bacillus thuringiensis* (Table 7.2).

Bt cotton has been genetically adapted by the accumulation of one or more genes from general soil bacteria, *Bacillus thuringiensis*. These genes produce insecticidal proteins, and therefore, genetically transformed plants generate one or more toxins. Bollworms are responsible for 60–70% of damage to cotton plants. Boll guard I and Boll guard II exhibited a reduction in the number of damaged bolls of 61 and 95%, respectively, compared with the conventional variety (Estruch et al. 1996). *VIP 3A + Cry IAb* expressing line gives the maximum mortality of susceptible and resistant strain of *Heliothis virescens* as compared to individual toxin expressing line and

Table 7.2 Bt transgenic plants expressing genes for insect resistance

Crop	Gene(s) for insect resistance	Target insect	References
Tobacco	<i>Magi6 peptide</i>	<i>Spodoptera frugiperda</i>	Hernandez-Campuzano et al. (2009)
	<i>cry1Ac</i> and <i>cry3A</i>	<i>Helicoverpa armigera</i> Hubner	Yuan et al. (2017a, b)
	<i>cry1Ac</i> and <i>cry2A</i>	<i>Phthorimaea operculella</i> Zeller	Bakhsh et al. (2018)
	<i>SmchiC</i>	<i>Botrytis cinerea</i> and <i>S. frugiperda</i>	Navarro-González et al. (2019)
	<i>Arginine kinase</i>	<i>Helicoverpa armigera</i> Hubner	Ai et al. (2019)
	<i>Vigna mungo</i> protease inhibitor (<i>VmPI</i>)	<i>Spodoptera litura</i>	Mudiyappanayar and Koundal (2020)
Tomato	<i>Proteinase inhibitor 2 (Pin2)</i>	<i>Tuta absoluta</i> (Meyrick)	Hamza et al. (2018)
	<i>cry2AX1</i>	<i>H. armigera</i> and <i>S. litura</i>	Sushmitha et al. (2018)
Potato	<i>cry1Ab</i>	<i>P. operculella</i> Zeller	Salehian et al. (2021a)
	<i>cry3A</i>	Colorado potato beetle	Salehian et al. (2021b)
Sugarcane	<i>Vip3A</i>	<i>Chilo infuscatellus</i>	Riaz et al. (2020)
Maize	<i>Cry1Ab/Cry2Aj</i>	<i>Ostrinia furnacalis</i> , <i>H. armigera</i> , and <i>Mythimna separata</i>	Liu et al. (2018)
	<i>Cry1Ab</i> , <i>Vip3Aa20</i>	<i>S. frugiperda</i>	Eghrari et al. (2021)
Rice	<i>cry1Ac</i> and <i>CpTI</i>	<i>Chilo suppressalis</i> , <i>Cnaphalocrocis medinalis</i> , and <i>Scirpophaga incertulas</i>	Han et al. (2008)
	<i>Maize proteinase inhibitor</i> and <i>potato carboxypeptidase inhibitor</i> fusion gene	<i>C. suppressalis</i>	Quilis et al. (2014)
Rice	<i>miR-14</i>	<i>C. suppressalis</i>	He et al. (2019)
Rice	<i>Asal</i>	<i>Sogatella furcifera</i> (WBPH), <i>Nephotettix</i> sp. (GLH), and <i>Nilaparvata lugens</i> (BPH)	Yarasi et al. (2008)
	<i>Asal</i> and <i>Galanthus nivalis</i> (<i>gna</i>) lectin genes	<i>S. furcifera</i> , <i>Nephotettix</i> sp., and <i>Nilaparvata lugens</i> (BPH)	Bharathi et al. (2011)
	<i>Dioscorea batatas</i> tuber lectin 1 (DB1)	<i>N. lugens</i>	Yoshimura et al. (2012)
	<i>Asal</i>	<i>N. lugens</i>	Chandrasekhar et al. (2014)
	<i>Cry1Ac::Asal</i>	<i>S. incertulas</i> , <i>C. medinalis</i> , and <i>N. lugens</i>	Boddupally et al. (2018)
	<i>Cry1Ab</i> and <i>Vip3A</i> fusion protein	<i>C. suppressalis</i> and <i>C. medinalis</i>	Xu et al. (2018a, b)

non-*Bt* line. *Bt* is very specific to particular insect pests and does not have any direct effect on any of the nontargeted beneficial insects. *Bt* rice provides resistance against various stem borers such as the following: striped stem borer (*Chilo suppressalis*), yellow stem borer (*Scirpophaga incertulas*), and pink stem borer (*Sesamia inferens*). More than 70 transgenic *Bt* rice lines of three selected cultivars, IR64, Pusa Basmati-1, and Karnal local, have been produced using the artificial shortened *Bt* gene, *cryIAc*. The *Bt* brinjal provides resistance against brinjal shoot and fruit borer. The first transgenic brinjal carried a synthetic *Bt-cryIAb* gene. At all locations, the *Bt* variety (MHB *Bt*) had significantly less brinjal fruit and shoot borer larvae and percent fruit damage. The transgenic *Bt* tomato expressing *CryIAb* protein, *CpTi* gene, etc. is effective against *Helicoverpa armigera*. Leaf-specific overexpression of the potato PI-II and carboxypeptidase inhibitors (PCI) results in resistance to *Heliothis obsoleta* and *Liriomyza trifolii* larvae in homozygote tomato lines expressing high levels of the transgenes. The transgenic sugarcane lines were generated expressing *Vip3A* toxin driven by polyubiquitin promoter for resistance against sugarcane stem borer. A direct correlation was observed between the *Vip3A* protein and *Vip3A* transgene expression in the transgenic sugarcane lines. In in vitro insect bioassay on V1, *Vip3A* transgenic sugarcane lines exhibited high resistance to *C. infuscatellus* with up to 100% mortality compared to the control sugarcane line. Thus, a single copy insertion of the *Vip3A* gene in transgenic sugarcane lines renders them resistant to borer, and these lines can be potentially used for the generation of insect-resistant transgenic sugarcane and could also be employed in gene pyramiding with *Bt* toxin to prolong resistance (Riaz et al. 2020).

Han et al. (2008) reported genetically modified rice lines containing *cryIAc* and *CptI* (cowpea trypsin inhibitor) to provide resistance against *Chilo suppressalis*, *Cnaphalocrocis medinalis*, and *Scirpophaga incertulas* pests for rice. The transgenics so developed reveals fluctuation in disease reaction toward the survival of *Sesamia inferens* (Pink Stem borer) larvae. Thus, further investigations were devised to delay its population density. Quilis et al. (2014) explained the role of proteinase inhibitors including maize proteinase inhibitor (MPI) and potato carboxypeptidase inhibitor (PCI) in insect resistance. Their fusion, followed by an introduction to rice plants, revealed a reduction in larval weight of *C. suppressalis* (striped stem borer), which is a major pest of rice. Also, the plants expressing *mpi-pci* fusion gene display enhanced resistance against *Magnaporthe oryzae*, the causal organism for rice blast. Thus, the fusion gene was reported to provide resistance for insects and pathogens as well in rice. He et al. (2019) demonstrated the transgenic lines with overexpressing *miR-14*, an insect-specific mRNA leading to the death of striped stem borer individuals. The *miR-14* has been reported to regulate metamorphosis in a variety of insects (Jayachandran et al. 2013; Liu et al. 2013; Varghese and Cohen 2007). Its overexpression resulted in interference with normal metamorphosis development of the insect by eliminating the functions of ecdysone after molting. Developing transgenic insect-resistant rice lines using miRNA significantly broadens the scope of target genes for pest control. Yarasi et al. (2008) reported the introduction of *Allium sativum* leaf lectin gene (*asal*) into indica rice cultivars susceptible to brown planthopper (BPH), green leafhopper (GLH), and white-backed planthopper (WBPH).

The calli were cocultivated with *Agrobacterium* comprising of pSB111 vector harboring *asal*, along with the herbicide resistance gene *bar*, under the control of CaMV35S promoter. The bioassay involving the expression of foreign gene reveals entomotoxic effects on BPH, GLH, and WBPH insects, with their decreasing survival, development, and fecundity of the insects. Also, the *asal* transgenic rice lines are a promising source of resistant cultivars. Among the sap-sucking pests, Bharathi et al. (2011) demonstrated the positive correlation of transgenic rice plants bearing pyramided *asal* and *gna* (*Galanthus nivalis*) lectin genes with the enhanced resistance conferred by the plant. Against BPH, transgenic lines have been developed, harboring *Dioscorea batatas* tuber lectin 1, and *asal* gene shows a high level of resistance against *Nilaparvata lugens* independently reported by Yoshimura et al. (2012) and Chandrasekhar et al. (2014). Boddupally et al. (2018) reported transgenic rice plants with Cry1Ac: ASAL fusion protein to provide resistance against the yellow stem borer (YSB), leaf folder (LF), and brown planthopper (BPH). The bioassays revealed 100%, 80–100%, and 70–80% mortality rate of pests of YSB, LF, and BPH, respectively. The study implied the enhanced efficacy of *Cry1Ac::Asal* fusion protein in minimizing pest population and providing insect resistance. Similarly, Xu et al. (2018a, b) reported the expression of the fusion protein of *Cry1Ab* and *Vip3A* protein in transgenic rice lines displayed efficient resistance against two major pests, viz., *C. suppressalis* and *C. medinalis*. Henceforth, these studies imply the role of transgenic rice plants harbors the significant potential for insect resistance management following various tissue culture and genetic engineering protocols.

7.3.2 Marker-Assisted Selection (MAS)

Locating and identifying genes of interest responsible for resistance is crucial for breeding insect-resistant varieties. The molecular marker-assisted selection of crops is one of the most fundamental applications of biotech tools. This progress has been facilitated by the construction of high-density genetic maps of certain plants and insects. Researchers have utilized molecular markers in crops linked to genes expressing resistance to several major insect pests. Molecular markers have been effectively applied for rice improvement. The main advantages of molecular markers include consistency, biosafety, time-saving, and efficient and accurate selection of complex traits (Jena and Mackill 2008). Application of molecular markers includes selecting the plants harboring specific genomic regions responsible for the expression of traits of interest (Das et al. 2017). The identified molecular markers are either linked to a single major gene for resistance or a group of loci controlling the expression of quantitative resistance known as quantitative trait loci (QTL). The first known case of QTL mapping for plant resistance to insects was in tomato, *Lycopersicon esculentum* Mill. The wild species of tomato, *L. hirsutum* f. *glabratum*, conferring resistance to arthropod pests had a principal toxic factor, viz., 2-tridecanone (2-TD). A mapping population of 74 F₂ individuals was evaluated for the

amount of 2-TD, and the marker loci on three different linkage groups were found associated with expression levels of 2-TD. In case of yellow stem borer (YSB) resistance, the detection of major quantitative trait loci could be of considerable value for insect resistance breeding programs, since their incorporation in susceptible genotypes permits a direct increase of the resistance level in the improved genotypes. Identification of markers associated with YSB resistance facilitates selection in applied breeding given the inherent difficulties in field-based screening for this pest. Linkage analysis with the F_2 phenotypic scores and RAPD data revealed that the RAPD markers K6695, C1320, and AH5660 were at a distance of 12.8 cM, 15.2 cM, and 14.9 cM, respectively, from the gene (s) of interest (Kammar and Nitin 2019).

At present, considerable attention has been focused on the resourceful wild species of rice for breeding purposes. The genus *Oryza* harbors 22 wild and 2 cultivated species. Among these, wild accessions represent an exclusive collection of rich germplasm bearing huge potential in crop improvement. Khush states that cultivated and wild species belong to different categories of genome, viz., AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK. Wide hybridization has been successfully applied since many years for providing resistance against various biotic and abiotic stresses in rice. It has been used to delimit the genotypes possessing exclusive properties for providing resistance, and thus selection of such genomes allows precise introgression for disease resistance. We will discuss some of the examples in the next paragraph.

The wild relative of rice, *O. australiensis* (accession 100,882), belonging to the EE genome displayed strong resistance and thus serves as a potential source of BPH resistance development. The *BPH10* and *BPH18* identified from *O. australiensis* harbor resistance to four biotypes of BPH, both belonging to the long arm of chromosome 12. Also, another QTL named *qBPH4.2* was found on the short arm of chromosome 4 and narrowed down to a 300 kb genomic region of the Nipponbare genome bracketed by RM261 and S1 markers (Hu et al. 2015a). *O. officinalis* has been found a significantly important source for BPH resistance comprising of *bph11*, *BPH12*, *BPH13*, *BPH14*, *BPH15*, *qBPH3*, and *qBPH4*. This wild species has been reported for the successful identification and introgression of various resistance gene(s)/QTLs WBPH7, WBPH8, *qSBPH3d*, *qSBPH7a*, and *qSBPH12b* against other planthoppers, viz., WBPH and SBPH. *O. rufipogon* stands as a progenitor of present-day cultivated rice possessing enriched genetic diversity and, thus, a significant reservoir for crop improvement programs in rice. This wild relative harbors diverse QTLs contributing tolerance toward various biotic and abiotic stresses (Ma et al. 2015; Vaughan et al. 2003; Xiao et al. 1998). BPH resistance from *O. minuta* belonging to BBCC genome has been successfully transferred to cultivated rice, henceforth responsible for providing a wide spectrum BPH resistance. Three dominant genes *BPH20*, *BPH21*, and *BPH23* have been reported for successful introgression from *O. minuta*. Also, *O. glaberrima* belonging to the cultivated rice category has been reported as a resistance source for BPH, GRH, and GLH. Apart from the usefulness of *O. nivara* genome against various abiotic

stresses, it has been successfully used to derive BPH resistance in the form of *BPH34* gene.

Collard and Mackill (2007) have reviewed the application of molecular markers in various rice improvement programs with superior advantages of molecular markers in terms of time, consistency, biosafety efficiency and accuracy. A diverse set of DNA markers have been effectively employed to identify resistance gene(s)/QTLs following MAS for integrating different resistance gene(s)/QTLs into the rice cultivars lacking the desired disease tolerance traits. Various genes and QTLs were identified from a wide rice germplasm worldwide against BPH, WBPH, SBPH, gall midge, green rice leafhopper, green leafhopper, and rice leaf folder for developing resistant varieties (Table 7.3).

Table 7.3 Details of the donor resources along with linked markers used in MAS

Source	Gene (s)/QTLs name	Chr	Linked markers	References
Cheongcheongbyeo	<i>BPH1</i>	12L	pBPH4-14	Cha et al. (2008)
ASD7	<i>bph2</i>	12L	RM1246-463	Sun et al. (2006)
Rathu Heenati	<i>BPH3, BPH17</i>	6S, 4S	RM1929-8072, RM8213-5953	Jairin et al. (2007b) Sun et al. (2005)
Babawee	<i>bph4</i>	6S	RM589-586	Jairin et al. (2010)
ARC10550	<i>bph5</i>	–	–	Khush et al. (1985)
Swarnalata	<i>BPH6</i>	4L	RM16994-119	Kabir and Khush (1988), Qiu et al. (2010)
T12	<i>bph7</i>	12L	RM3448-313	Kabir and Khush (1988), Qiu et al. (2014)
Chin Saba	<i>bph8</i>	–	–	Nemoto et al. (1989)
Pokkali	<i>BPH9</i>	12L	InD2-RsaI	Nemoto et al. (1989), Zhao et al. (2016)
<i>O. australiensis</i>	<i>BPH10, BPH18, qBPH4.2</i>	12L, 12L, 4S	RG457-CDO459, BIM3-BN162, RM261-XC4-27	Ishii et al. (1994) Ji et al. (2016) Hu et al. (2015a)
<i>O. officinalis</i>	<i>bph11, BPH12, BPH13, BPH14, BPH15</i>	3L, 4S, 3S, 3L, 4S	G1318, RM16459-1305, RZ892-RG191, S M1-G1318, RG1-RG2	Hirabayashi et al. (1998) Qiu et al. (2012) Renganayaki et al. (2002) Du et al. (2009) Yang et al. (2004)
	<i>qBPH3, qBPH4</i>	3, 4	t6-f3, P17-xc4-27	Hu et al. (2015b)
	<i>WBPH7, WBPH8</i>	3, 4	R1925-G1318, R288-S11182	Tan et al. (2004)
	<i>qSBPH3d, qSBPH7a, qSBPH12b</i>	3, 7, 12	RM218-745, RM7012-6338, RM463-6256	Zhang et al. (2014)

(continued)

Table 7.3 (continued)

Source	Gene (s)/QTLs name	Chr	Linked markers	References
M1635–7	<i>BPH16</i>	12	RM6732-R10289	Hirabayashi et al. (2004)
<i>O. rufipogon</i>	<i>bph18(t)</i> , <i>bph19(t)</i> , <i>bph22(t)</i> , <i>bph23(t)</i> , <i>bph24(t)</i> , <i>BPH27</i> , <i>bph29</i> , <i>bph30</i> , <i>BPH36</i>	4L, 12, 4, 8, –, 4L, 6S, 10S, 4S	RM273-6506, RM17, RM8212-261, RM2655-3572, –, RM16846-16853, BYL8-BID2, RM222-244, RM16465-16502	Li et al. (2006) Li et al. (2006) Hou et al. (2011) Hou et al. (2011) Deen et al. (2010) Huang et al. (2013) Wang et al. (2015) Yang et al. (2012) Li et al. (2019)
	<i>qWPH2</i> , <i>qWBPH5</i> , <i>qWBPH9</i>	2, 5, 9	RM1285-555, RM3870-RZ70, RG451-RM245	Chen et al. (2010a, b)
	<i>GRH5</i>	8	RM3754-3761	Fujita et al. (2006)
AS20–1	<i>bph19(t)</i>	3S	RM6308, RM3134	Chen et al. (2006)
<i>O. minuta</i>	<i>BPH20(t)</i> , <i>BPH21(t)</i> , <i>BPH23(t)</i>	4S, 12L,	B42:B4, M510, RM5953, S12094A-B122	Rahman et al. (2009) Rahman et al. (2009) Ram et al. (2010)
<i>O. glaberrima</i>	<i>BPH22(t)</i>	–	–	Ram et al. (2010)
	<i>qGRH9</i>	9	RM215-RM2482	Fujita et al. (2010)
ADR52	<i>bph25</i> , <i>BPH26</i>	6S, 12L	S00310-RM8101, DS72B4-DS173B	Myint et al. (2012) Tamura et al. (2014)
	<i>WBPH3</i>	–	–	Hernandez and Khush (1981)
Balamawee	<i>BPH27(t)</i>	4L	Q52, Q20	He et al. (2013)
DV85	<i>BPH28(t)/QBPH11</i>	1L	InDel55, InDel66	Wu et al. (2014)
AC-1613	<i>BPH30</i>	4S	SSR28, SSR69	Wang et al. (2018)
CR2711–76	<i>BPH31</i>	3L	PA26, RM2334	Prahalada et al. (2017)
PTB33	<i>BPH32</i>	6S	RM19291, RM8072	Ren et al. (2016)
KOLAYAL	<i>BPH33</i>	4S	H99, H101	Hu et al. (2018)
<i>O. nivara</i>	<i>BPH34</i>	4L	RM16994, RM17007	Kumar et al. (2018)
IR64	<i>BPH37</i>	1	RM302, YM35	Yang et al. (2019)
Khazar	<i>BPH38(t)</i>	1L	SNP-693369, id10112165	Balachiranjeevi et al. (2019)
Salkathi	<i>qBPH4.3</i>	4	RM551, RM335	Mohanty et al. (2017)
	<i>qBPH4.4</i>	4	RM335, RM5633	
IR71033–121-15	<i>qBPH6(t)</i>	6	RM469, RM568	Jairin et al. (2007a)
Nagina 22	<i>WBPH1</i>	7	–	Sidhu et al. (1979)
ARC10239	<i>WBPH2</i>	6	RZ667	Angeles et al. (1981), Liu et al. (2002)

(continued)

Table 7.3 (continued)

Source	Gene (s)/QTLs name	Chr	Linked markers	References
Podiwi A8	<i>wbph4</i>	–	–	Hernandez and Khush (1981)
N'Diang Marie	<i>WBPH5</i>	–	–	Wu and Khush (1985)
Guiyigu	<i>WBPH6</i>	11	RM167	Li et al. (2004)
Sinna Sivappu	<i>wbph9(t)</i> , <i>wbph10(t)</i> , <i>wbph11(t)</i> , <i>WBPH12(t)</i>	6, 12, 4, 4	RM589-539, SSR12- 17.2-RM28487, RM3643-1223, RM16592-16649	Ramesh et al. (2014)
Asominori	<i>Ovc</i> , <i>qOVA-1-3</i> , <i>qOVA-4</i> , <i>qOVA-5-1</i> , <i>qOVA-5-2</i>	6, 1, 4, 5, 5	R2373-C946, XNpb346-C112, R1854, XNpb251-R3313, C1268	Yamasaki et al. (2003)
Chuanjiang 06	<i>qWL6</i>	6	M3, M5	Yang et al. (2014)
	<i>qRLF-3</i> , <i>qRLF-4</i> , <i>qRLF-8</i>	3, 4, 8	RM1022-7, RM3276-255, RM72-331	Rao et al. (2010)
IR54751	<i>qWBPH3.2</i> , <i>qWBPH11</i>	3, 11	InDel3-23- InDel3-26, DJ53973-SNP56	Fan et al. (2018)
Mudgo	<i>qSBPH2b</i> , <i>qSBPH3d</i> , <i>qSBPH12a</i>	2, 3, 12	RM29-5791, RM5442-3199, I12-17, RM3331	Duan et al. (2009)
Kasalath	<i>qSBPH2</i> , <i>qSBPH3</i> , <i>qSBPH8</i> , <i>qSBPH11</i>	2, 3, 8, 11	R712-R1843, C1135-C80, R1943-C390, G257-S2260	Duan et al. (2010)
N22	<i>qSBPH2</i> , <i>qSBPH3</i> , <i>qSBPH5</i> , <i>qSBPH7</i> , <i>qSBPH11</i>	2, 3, 5, 7, 11	RM263-1385, RM22-545, RM153-413, RM234-429, RM209-RM21	Wang et al. (2013)
9194	<i>qSBPH1</i> , <i>qSBPH5</i> , <i>qSBPH8</i> , <i>qSBPH9</i>	1, 5, 8, 9	RM3738-8236, RM18452-163, RM210-3845, RM257-160	Sun et al. (2017)
WR24	<i>qSBPH5</i> , <i>qSBPH7</i> , <i>qSBPH10</i>	5, 7, 10	Indel 5–11, RM3664, RM6403-234, RM25664-228	Xu et al. (2018b)
W1263	<i>GM1</i>	9S	RM444-219	Biradar et al. (2004)
Phalguna	<i>GM2</i>	4	RM241-317	Himabindu et al. (2007)
RP2068-18-3-5	<i>gm3</i>	4	RM17480- gm3SSR4	Sama et al. (2014)
Abhaya	<i>GM4</i>	8L	RM22551-22562	Divya et al. (2015)

(continued)

Table 7.3 (continued)

Source	Gene (s)/QTLs name	Chr	Linked markers	References
ARC5984	<i>GM5</i>	12	RM101-309	Dubey and Chandel (2010)
Duokang #1	<i>GM6</i>	4L	RG214-RG476	Katiyar et al. (2001)
RP2333-156-8	<i>GM7</i>	4	F8LB-SA598	Sardesai et al. (2002)
Aganni	<i>GM8</i>	8S	RM22685-22709	Divya et al. (2018)
Line9	<i>GM9</i>		–	Shrivastava et al. (2003)
BG 380-2	<i>GM10</i>	–	–	Kumar et al. (2005)
CR57-MR1523	<i>GM11</i>	12	RM28574-28706	Himabindu et al. (2010)
IR24	<i>GRH1</i>	5	R569-C309	Kadowaki et al. (2003)
DV85	<i>GRH2</i>	11	R2458-C50	Kadowaki et al. (2003)
Rantaj emas 2	<i>GRH3</i>	6	C288B-C133A	Saka et al. (2006)
DV85	<i>GRH4</i>	3	C1186-R2982	Kadowaki et al. (2003)
SML17, IRGC105715	<i>GRH6</i>	4	RM8213-C708	Fujita et al. (2004), Tamura et al. (2004)
Maddani Karuppan	<i>GLH 7</i>	–	–	Rezaul Karim and Pathak (1982)
DV85	<i>glh8</i>	–	–	Ghani and Khush (1998)
IR28	<i>GLH 9</i>	–	–	Angeles and Khush (1999)
IR36	<i>glh10</i>	–	–	Angeles and Khush (2000a)
IR20965-11-3-3	<i>GLH 11</i>	–	–	Angeles and Khush (2000b)
ARC10313	<i>GLH 12</i>	–	–	Angeles and Khush (2000b)
Asmaita	<i>GLH 13</i>	–	–	Angeles and Khush (2000b)
ARC11554	<i>GLH 14</i>	4	Y3635-RZ262	Sebastian et al. (1996)
Taichung Native 1	<i>qRLF-1</i>	1	RM3412-6716	Rao et al. (2010)
	<i>qRLF-2</i>	2	RM207-48	

7.3.3 Gene Pyramiding

Improved insect resistance has also been achieved through the employment of multiple resistance genes in a single plant, also known as gene stacking or gene pyramiding. Multiple insect-resistant genes stacking in the transgenic *Bt* crops have been employed to confer resistance to the insects and herbicides. The first transgenic *Bt* crop (cotton) with stacked genes, *Cry1Ac* and *Cry2Ab2*, registered for use in the USA in 2002, was Bollgard II. These stacked genes in the transgenic cotton have been very effective against the pink bollworms (*Pectinophora gossypiella*)

(Stefey et al. 2009). These genes (*CryIAc* and *CryIC*), also stacked in transgenic Bt broccoli, had the potential to delay resistance to the diamondback moth (*Plutella xylostella*) more effectively than the transgenic plants with single Bt gene (Zhao et al. 2003). Wang et al. (2017) developed LuoYang69 restorer line of 93–11, harboring two pyramided BPH resistance genes, *BPH6* and *BPH9*, using marker-assisted selection. The resultant line displays an enhanced resistance reaction toward BPH. He et al. (2020) reported pyramiding of *BPH3*, *BPH14*, *BPH18*, and *BPH32* resistance genes in Guang 8B rice variety. The study suggested additional increase in resistance level by the introduction of four genes. Venkanna et al. (2018) provided evidence for stacking three gall midge resistance genes—*Gm1*, *gm3*, and *Gm8*—in an improved line WGL-1068, developed as the F5 generation of the cross between Kavya (susceptible cultivar) and gall midge-resistant introgression line Samba Mahsuri. Apart from gall midge resistance, the improved line possesses high-yielding and fine-grain characters better than elite variety Kavya. Wang et al. (2017) developed LuoYang69 restorer line of 93-11 harboring two pyramided BPH resistance genes *BPH6* and *BPH9* using marker-assisted selection. The resultant line displays an enhanced resistance reaction towards BPH. He et al. (2020) reported pyramiding of *BPH3*, *BPH14*, *BPH18* and *BPH32* resistance genes in Guang 8B rice variety. The study suggested an additional increase in resistance level by the introduction of 4 genes. Venkana et al. (2018) provided evidence for stacking 3 gall midge resistance genes; *Gm1*, *gm3* and *Gm8* in an improved line WGL-1068 developed as F5 generation of the cross between Kavya (susceptible cultivar) with gall midge resistant introgression line Samba Mahsuri. Apart from gall midge resistance, the improved line possesses high yielding and fine-grain characters better than elite variety Kavya. Jena et al. (2017) developed 25 NILs, among which 16 lines belonged to multiple resistance gene combinations. Apart from these, multiple disease resistance programs have revolutionized breeding programs recently. Reinke et al. (2018) developed various moderately resistant lines, harboring brown planthopper, rice stripe virus, rice blast, and bacterial blight-resistant genes in different combinations. Following the marker-assisted selection, the MR lines selected were encompassing *BPH18*, *qSTV11^{SG}*, *Pib* and *Pik*, and *Xa40* or *Xa3* to provide stable resistance with effect on major agronomic traits. The pyramiding of genes harbors profound antibiosis reactions during BPH infestation as compared to single resistance gene bearing lines. This way, critically developed pyramided lines can act as a rich genetic source for breeding purposes in light of insect resistance (Plate 7.3).

7.3.4 Functional Genomics

Functional genomics emerges as an advanced field of biotechnology that has presented diverse platforms in agricultural research programs. Rice is considered as a model plant for functional genomics studies owing to its smaller genome, sequenced genome, vast transformation methodologies, and abundant germplasm availability (Jiang et al. 2012). Among the rice germplasm, the availability of wild relatives, rice

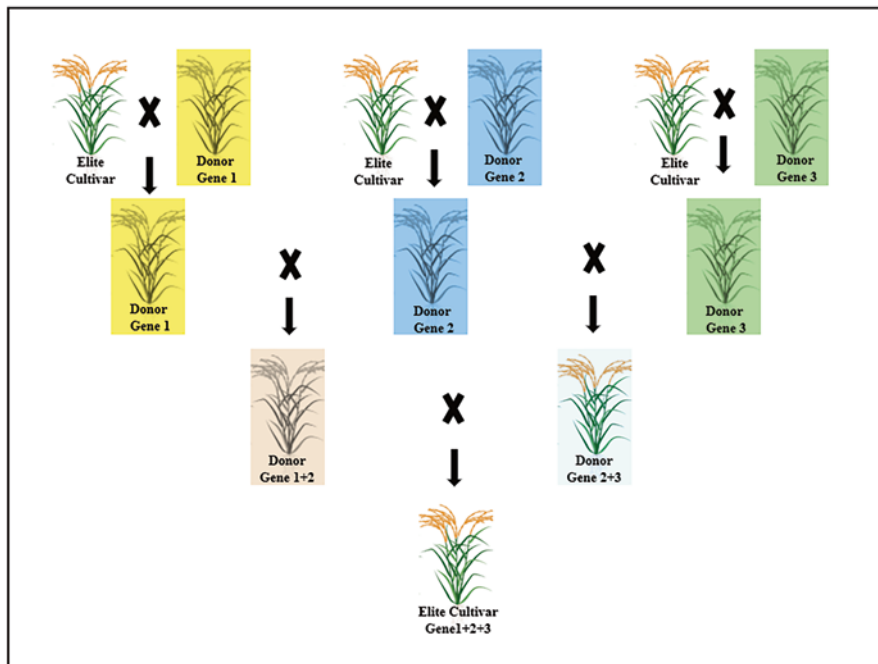


Plate 7.3 Gene pyramiding in elite rice cultivar with multiple insect resistance genes

mutant libraries and rice genome-based databases, opens new avenues for functional genomics studies relating to other crop plants as well. This field deals with the functional characterization of various genes in the genome, which is obtained through gain or loss of functions in plants. Wei and Chen (2018) presented a report focusing on the comparison of the basic helix-loop-helix transcription factors (bHLH) in *Arabidopsis*, rice, maize, and wheat. The comparative functional genomics studies were carried using available genome assembly databases. Among the family, different subfamilies confirmed their role in iron uptake, anther development, disease tolerance via different defense pathways, and secondary metabolite production. The resultant information regarding constitutive and differentially expressing bHLH can serve as a hub of its functional characterization in *Gramineae* species, thus contributing toward molecular breeding approaches. The fungus *Magnaporthe oryzae* is considered to secrete proteins that are responsible for disease reactions in rice plants. However, the functions of effector proteins are not explored in a way to enhance disease resistance. Guo et al. (2019) deduced the functional aspect of various proteins of the fungus, following transient expression assays of 98 in planta-expressed *M. oryzae*. The researcher devised eight novel proteins, MoCDIP6 to MoCDIP13, responsible for rice blast owing to death. Thus, similar studies can help to accelerate the understanding of mechanisms underlying the pathogenic infection, which in turn can be utilized as a key source for

developing resistant cultivars. Among the genes pertaining to host, Li et al. (2020) utilized the transgenic plants with insect-inducible promoters as an important strategy for resistance against the striped rice stem borer (*Chilo suppressalis*). This first reporter of SSB-inducible promoter states the upregulation of *hydroperoxide lyase* gene (*OsHPL2*) post insect feeding. Thus, cloning strategy was directed toward the promoter of this gene, devising the promoter and positive regulatory regions exhibiting SSB larval mortality. Thus, functional information related to the host as well as pathogen genes and promoters can serve as a potential source for accelerating the insect-resistant rice cultivars.

7.3.5 RNA Interference (RNAi)

Since the discovery that dsRNA can silence genes, RNAi has been developed as an effective tool for regulating gene expression (Vogel et al. 2019). This approach bears significant potential in the field of crop improvement due to its preferential target specificity and low negative environmental effect (Chung et al. 2021). RNAi or gene silencing has been used to inhibit virus replication in transgenic plants and has the potential to be developed commercially for insect management also. RNAi constructs directed toward targeting insect-derived genes are considered as a promising approach for agricultural pest control (Chung et al. 2021). Insect genes can be downregulated by injection of dsRNA or by oral administration of high concentrations of exogenously supplied dsRNA as part of an artificial diet, but a much more efficient method of delivering dsRNA is needed before RNAi technology can be used to control pests in the field (Mao et al. 2007; Bettencourt et al. 2002). Before now, a very sensitive RNAi response has been observed in the Western corn rootworm (WCR) *D. virgifera virgifera*, to oral administration of dsRNA and the first RNAi-based insecticides for the control of this insect have already been approved by the US Environmental Protection Agency (EPA). This plant-incorporated protectant (PIP) employs pyramid strategy where several different *Bt* proteins (crystalline toxins) and dsRNA targeting the WCR *Snf7* gene, will be expressed in the plant (Head et al. 2017). Contrarily, downregulation of *Snf7*, a gene that plays an essential role in protein trafficking, will also result in mortality (Bolognesi et al. 2012). So this integrated strategy is intended to target the insect while also reducing the chances for insects to develop resistance against the PIP (Head et al. 2017). As RNAi is a growing tool within the field of biotechnology, it will definitely show up as a strong insecticidal strategy for crop improvement (Kunte et al. 2019).

Insect genes that serve as a target for successful RNAi constructs include the following: gene encoding enzymes of basal insect metabolism, effectors responsible for plant defense suppression, detoxifying and digestive enzymes, genes involved in detoxification of defensive secondary metabolites of the hosts, etc. (Chung et al. 2021). He et al. (2019) reported the expression of artificial miRNAs in transgenic rice, providing profound resistance to *Chilo suppressalis* (rice stem borer). The course of action involved in the process includes high mortality and developmental

defects, owing to targeting the ecdysone receptor of insects. In addition, Kola et al. (2019) determined that the knockdown of acetylcholinesterase gene of *Scirpophaga incertulas* (rice yellow stem borer) using dsRNA construct in transgenic rice leads to reduced larval weight. Thus, the genome of insects and pests carrying specific genes facilitating the disease occurrence can be targeted by different constructs following specific delivery methodologies to cure the potential spread of disease. Recently, nanoparticles, such as chitosan, liposomes, and cationic dendrimers, offer advantages in delivering dsRNA/small interfering (si)RNA (siRNA) to improve RNAi efficiency, thus promoting the development and practice of RNAi-based insect management strategies (Yan et al. 2021) (Table 7.4).

Table 7.4 RNAi for insect resistance

Target pest	Target gene	Function	Effect	References
BPH	<i>Entomomyces delphacidicola arginine-succinate lyase (EdArg4)</i>	Arginine biosynthesis	Delayed nymphal development, thickened wings, enlarged antennae, legs, and anal tubes in adults	Yuan et al. (2017a, b)
	<i>Trehalase (TRE)</i>	Wing bud formation and molting	Deformed wings	Zhang et al. (2017)
	<i>20-Hydroxyecdysone</i>	Molting and metamorphosis	Decrease in transcript level, reduction in fecundity	Yu et al. (2014)
	<i>Vacuolar ATP synthase subunit E (V-ATPase-E, 21E01)</i>	Membrane transporter binding protein	Decreased expression of target gene	Li et al. (2011)
	<i>Hexose transporter, carboxypeptidase, trypsin like serine protease</i>	Transport of glucose, hydrolysis of protein	Depletion in transcript level and no effect on larval survival	Zha et al. (2011)
	<i>Trehalose phosphate synthase (TPS)</i>	Production of trehalose-6-phosphate	Decreased survival rate	Chen et al. (2010a, b)
Yellow stem borer	<i>Cytochrome P450 derivative (CYP6)</i> and <i>aminopeptidase N (APN)</i>	Metabolism of insecticides and protein digestion	Detrimental effect on larval growth and development	Kola et al. (2016)
WBPH	<i>Halloween gene disembodied (dib)</i>	Encodes cytochrome P450 monooxygenase CYP302A1 (22-hydroxylase) which plays a role in ecdysteroidogenesis	Reduction in <i>dib</i> and <i>EcR</i> (ecdysone receptor) transcript, development and survival of nymphs was impaired	Wan et al. (2014)

7.3.6 CRISPR Cas

Clustered regularly interspaced short palindromic repeats (CRISPR) and the CRISPR-associated gene *Cas9* represent a valuable system for specific editing of genes in diverse species. So far, genome editing has been demonstrated in model species, like *Arabidopsis*, as well as important crops, like rice, wheat, maize, etc. Genome editing system has unfolded several possibilities that enable precise and efficient targeted modifications in diverse agronomic traits, including durable resistance against insect pests and pathogens. CRISPR/Cas9 mediated editing has been used to generate insect- and pathogen-resistant crops by knocking out of host susceptibility genes, exploiting the effector-target interaction, engineering synthetic immune receptor eliciting broad-spectrum resistance, etc. Modification of insect genomes through CRISPR/Cas9 has been used either to create gene drive or to counteract resistance to various insecticides. Lu et al. (2018) reported the knock-down of *CYP71A1* (encoding tryptamine 5-hydroxylase) following CRISPR/Cas9 methodology, leading to an increased level of salicylic acid and decreased serotonin levels, thereby providing resistance against BPH in rice (Du et al. 2020). Further, expressing insecticidal bacterial genes, anti-nutritional proteins like protease inhibitors, lectins, host-delivered RNAi and the modification of defense-signaling pathways can be utilized for insect resistance (Bisht et al. 2019). The experiment conducted by Li et al. (2020) demonstrates five genes, *OsWRKY2*, *OsWRKY14*, *OsWRKY26*, *OsWRKY69*, and *OsWRKY93*, induced in response to *Magnaporthe oryzae* infection. The increased transcript level of *OsWRKY93* pertains to resistance conferred against *M. oryzae* in rice. The results were validated with the development of *oswrky93-1* CRISPR knockout mutant's susceptibility toward *M. oryzae* infection. These results clearly indicate that the senescence-inducible gene *OsWRKY93* is also a positive regulator of the defense response and can be utilized for attaining resistance against *M. oryzae*.

7.3.7 Proteomics and Metabolomics

Proteomics and metabolomics are the two new emerging omics technologies that have the potential to provide complete information on the biological and metabolic processes of an organism. These technologies have been successfully exploring the differences in gene expression, protein and metabolite abundance, and modification of the posttranslational protein and providing a different level of views for the cellular processes that occur in cells. A proteomics and metabolomics study was executed on four wheat cultivars against wheat stem sawfly (WSS) infestation. Using liquid chromatography-mass spectrometry, the reported cultivars were infested with WSS, and variations in stem proteins and metabolites were detected. The proteome

included 1830 proteins, contributing in five major biological processes, i.e., metabolic processes and stimuli response, metabolome spanning eight chemical super classes of alkaloids, benzenoids, and lipids. Following infestation, the varieties under study showed molecular response to WSS. The data validated variation in the wheat stem molecular response against WSS infestation that supports different breeding approaches for insect resistance in wheat (Lavergne et al. 2020).

Henceforth, studying the proteome and metabolome level of the plant is critical to understand the host response under biotic stress. Erb and Kliebenstein (2020) proposed that metabolites involved in defense reactions in rice include volatile indole, glucosinolates, benzoxazinoids, phenylpropanoid phytoalexins, diterpenoid phytoalexins, and phenylamine. Kang et al. (2019) conducted a comparative metabolomics analysis to reveal the differences in metabolite profiles of susceptible rice cultivar (TN1) and two resistant cultivars (IR36 and IR56) in response to BPH infestations. The gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) investigations reveal the differentially expressed metabolites that included the defense-related metabolites, viz., induction of cyanoamino acids and lipid metabolism in IR36 and changes in thiamine, taurine, and hypotaurine metabolism in IR56 during BPH infection. Apart from these, quercetin and spermidine content were elevated in TN1 and IR36 owing harm to BPH insects. Thus, differences in metabolite profile upon BPH infestations reveal the metabolic mechanism and pathways that can be exploited as a resource for effective pest control. Furthermore, Uawisetwathana et al. (2019) reported the increment in flavonoid glycosides level subjected to resistant reaction in rice against BPH. Apart from BPH, Cheah et al. (2020) reported the proteomic analysis aided by SWATH-MS to identify the proteome profile of Qingliu and TN1 under the attack of *Cnaphalocrocis medinalis*. The results described the overrepresentation of proteins involved in photosynthesis, amino acid metabolic processes, and processes involving secondary metabolites. Also, Dong et al. (2017) reported comparative analysis of protein profiles in the leaf sheath of Pf9279-4 and 02428 representing small brown planthopper (*Laodelphax striatellus* Fallén, Homoptera, Delphacidae)-resistant and susceptible genotypes. The protein expression profile of both genotypes reveals that proteins induced by SBPH feeding were majorly employed in photosynthesis, cell wall-related proteins, amino acid metabolism, stress response, energy metabolism, carbohydrate metabolic process, and transcriptional regulation. The resistant genotype revealed a higher level of superoxide dismutase and glutathione and a defense pathway governed by salicylic acid. Liu et al. (2016) revealed that resistant rice plants infected with *Cnaphalocrocis medinalis* and *Chilo suppressalis*, respectively, displayed induction of photosynthesis that activated the biosynthesis of certain amino acids and metabolites. The differential proteome and metabolome levels among the host-adapted and non-adapted pathogens infer the knowledge regarding the adaptability of pathogens in terms of rice resistance at the proteomics and metabolomics level.

7.4 Integrated Pest Management

Biotechnology in the context of insect pest management can provide controlled, specific, and early by-products for insect pest control, which will have more substantial implications for agriculture than simply improved IPM. Currently, biotechnology is being applied for the precise characterization of insect pest species as well as identification and characterization of novel genes from the host for significant insect resistance. The development of insect-resistant crop varieties suppressing insect pest abundance with minimal environmental loss is the main aim of insect pest management. Till now, many resistant genes have been identified from host plants and diverse exotic sources and inserted into microorganisms and crop plants to confer resistance to insect pests and have improved understanding of gene action and metabolic pathways. For example, the insecticidal *Cry* family genes from *Bacillus thuringiensis* expressing insecticidal *Cry* proteins (*Bt* toxins) are deployed against an equally vast range of insect pest species. A parallel search on other possible non-*Bt* insect-resistant proteins has identified a large number of genes, holding great potential to interfere with the development and nutrition of different insect pests. Important gene(s), which have attracted scientific attention for rendering similar insect resistance potential in different crop plants, are *vegetative insecticidal proteins (VIPs)* (produced by different bacterial species including *B. cereus* and *B. thuringiensis*, toxic to coleopteran and lepidopteran insects), biotin-binding proteins (avidin and streptavidin are insect growth-inhibiting proteins whose genes could potentially be expressed in plants to provide inbuilt resistance to insect pests.), chitinases (target chitin in the peritrophic membrane of the midgut, causing a reduction in survival and growth), proteinase inhibitors (interfere with the activity of midgut proteinases, causing nutritional limitations), bean α -amylase inhibitors (α -amylase inhibitor peptides from some legume seeds impart resistance to coleopteran seed weevils), plant lectins (constitute direct defense responses in plants against attack by phytophagous insects), and scorpion and spider Venoms (exert a neurotoxic effect in other insect species) (Gupta and Jindal 2014). Biotechnology, as applied to insects now, provides ample opportunities for the identification and utilization of new genes to open a new field for their exploitation in effective insect pest control. The future prospects for biotechnological applications to mediate crop protection against insects using novel approaches along with wide-scale adoption of genetically modified biotech crops worldwide have formed high potential of biotechnology for the improvement of crops.

7.5 Conclusion

Biotechnology has been central to the acceleration of crop improvement over the last two decades. Among the most impactful biotechnology-derived traits, insect-pest resistance has greatly contributed to the worldwide increase in agricultural

productivity and stabilization of food security. The existence of multiple insect pests simultaneously in the field becomes inopportune for the plant survival; thus, incorporation of broad-spectrum resistance genes is required to minimize the loss and investment of rice farmers in the future. The methodologies in biotechnology and molecular biology serve as tools in developing resistant varieties to hasten crop improvement. For the past decades, rapid technological advances have made the discovery and analysis of plant and insect genomes accessible for research and improvement. Diverse techniques, like genetic engineering, wide hybridization, MAS, RNAi, and CRISPR, have provided a boost in identifying putative insect effectors, cloning insect resistance genes, selecting traits that are difficult to measure and observe, and revealing the key components of plant-insect resistance signals. Advances in biotechnology techniques like MAS have already been used to pyramid multiple insect resistance genes to cultivate durable, broad-spectrum insect resistance rice. At the same time, the new emerging technologies such as CRISPR/Cas9 gene editing to convert insect-susceptible alleles to insect resistance alleles, *in vivo*, provide the potential to design crops that can be patched in real time to combat evolving pests. Recent development in RNAi has provided an efficient means for identification and functional analysis of new plant genes, which are specifically expressed in response to the insect-pest attacks. Furthermore, the emerging biotechnological technologies will enhance the insect resistance and regulate plant immunity in rice varieties. However, in order to fully exploit the enormous potential of biotechnology, appropriate biosafety regulatory frameworks need to be effectively implemented. These integrated approaches can commute the dynamic threat of insects and ably contribute to sustainable development.

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