Increasing Rice Grain Yield Under Biotic Stresses: Mutagenesis, Transgenics and Genomics Approaches

Aamir Raina and Samiullah Khan

Abstract

Rice (*Oryza sativa* L.) is the most important source of staple food to a major portion of human population. The production of rice is reduced by several kinds of biotic stresses. The main biotic stresses that severely hamper the rice production include viruses, bacteria fungi, nematodes and insects. Different conventional and modern biotechnological approaches have been implemented to combat the devastating effect of different biotic stresses on the rice production. Conventional approaches such as hybridisation have led to the development of stress-tolerant varieties. The modern biotechnological approaches such as genomics and transgenics have led to the identification of genes that confer tolerance to stresses followed by its insertion into the rice plants with the aim of decreasing the yield loss incurred by the different stresses. Mutagenesis, genomics and transgenic approaches have been very effective in developing varieties with improved tolerance to various stress factors. Here we review the creation of rice varieties with improved yield under different biotic stress, using mutagenesis, transgenics and genomics approaches.

Keywords

Biotic stresses · Breeding techniques · Yield · Stress tolerance · Mutagenesis · Transgenics · Genomics

A. Raina (✉)
Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

Botany Section, Women's College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

S. Khan
Mutation Breeding Laboratory, Department of Botany, Aligarh Muslim University, Aligarh, Uttar Pradesh, India

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1 Introduction

Oryza sativa commonly known as rice belongs to family Poaceae with more than 80,000 accessions maintained at International Rice Research Institute (IRRI), Philippines. It is an ancient staple food with the origin of centre in southern and south-western tropical Asia and origin of domestication in India and China (Vavilov 1926; Ding 1957). *Oryza sativa* is considered as the main cultivated species of rice across the globe. It is one of the main cereal grains and source of food for more than 3.5 billion people, grown on 145 million ha in more than 110 countries (IRRI, Africa Rice and CIAT 2010; Heinrichs 1994). With the rapid increase in human population which is expected to increase up to 9 billion, by the end of 2050, rice production must increase by substantial amount. Increasing population and economic development have been posing a growing pressure for increase in rice production (Zhang 2007). This increase in rice production is a challenging task due to several factors such as decrease in rice lands, depleting water resources, erratic rainfalls and climate change. Further the overall yield of rice is sternly reduced by several biotic stress factors including virus, bacteria, fungi, insect pest, nematodes and diseases (Shamim and Singh 2017). To meet the challenges new rice varieties with improved yield and better tolerance to biotic stresses should be developed. This can be achieved by making the use of modern biotechnological approaches. Rice production needs to increase via biotechnological techniques with the objective of improving yield, resistance to biotic stresses and grain quality (Shamim and Singh 2017). Here we review the current progress in the field of mutagenesis, transgenics and genomics for the development of rice varieties that are resistant to wide range of biotic stresses.

1.1 Biotic Stresses

Rice production is negatively impacted by a wide range of biotic stresses that cause dreadful diseases and significantly decrease the overall productivity by 30% (Yadav and Srivastava 2017). Biotic stresses that devastate the rice production include virus, bacteria, fungi, nematode and insect pests (Ling 1980). Conventional breeding approaches have been implemented to combat the effects of biotic stresses but all such approaches have some limitations. Few limitations include cumbersome, laborious and huge time taken usually 10 years for the release of varieties with improved tolerance and yielding potential. Different causative agents results in the occurrence of dreadful diseases that incur huge loss in both production and economic values. On an average 10–15% of annual yield is lost due to different rice diseases across the world. In India different causative agents cause a substantial decrease in rice production that range from 6 to 60% depending upon the growth stage, variety and timing of occurrence of stress (Ou 1985; Singh et al. 1977). Hence, proper disease management could be useful to enhance production and recovery of yield losses. The rice diseases that have incurred a huge economic losses are rice blast (causative agent: *Magnaporthe grisea*), seedling blight (causative agent:

Pseudomonas plantarii), sheath blight (causative agent: *Rhizoctonia solani*), bacterial blight (causative agent: *Xanthomonas oryzae*), bacterial brown stripe (*Pseudomonas avenae* and *P. syringae* pv. *panici*), tungro virus disease and false smut (FS) (causative agent: *Ustilaginoidea virens*). Modern breeding approaches such as mutagenesis, transgenics and genomics have proven promising techniques in developing varieties with improved biotic stress tolerance. Among the techniques RNA interference (RNAi)-induced gene silencing has proven as an effective and efficient technique to engineer resistant plants to various kinds of biotic stresses and to mediate management of rice diseases. Rice is continuously affected by various organisms from insects to bacteria. A study estimated an annual loss of yield ranging from 120 to 200 mt due to wide range of causative agents in rice lands of tropical Asia (Willocquet et al. 2004). Biotic stresses that affect the rice production are discussed in this chapter.

1.2 Viral Diseases

Viral diseases represent a severe threat to rice production in Southeast Asian countries. The most common symptoms include abnormal growth and colour changes on leaves from green to yellow to white/orange. The teratological symptoms are stunted growth, reduced tillers, twisting, leaf rolling, gall formation on leaves and necrotic spots on culms. The rice yellow mottle virus (RYMV) is one of the most detrimental virus infecting rice. Rice tungro disease (RTD) is another damaging disease of rice, widespread in South and Southeast Asia. RTD incurs an annual loss of about 109 US dollars in the affected countries (Herdt 1991) and about 2% reduction in overall production in India (Muralidharan et al. 2003). A DNA virus, viz. *Rice tungro bacilliform virus* (RTBV), and an RNA virus, viz. *Rice tungro spherical virus* (RTSV), are causative agents of rice tungro disease. The initial reports of appearance of RTD in India came into notice in the late 1960s (Raychaudhury et al. 1967a, b), and thereafter extensive studies were carried out for its management (Rivera and Ou 1965). At present new information on theoretical and practical aspects and diagnostic techniques consistently regarding the causative agents, pathogenesis, vector transmission and resistance genes of RTD became available and sophisticated over time (Azzam and Chancellor 2002). In general plant viruses are transmitted mechanically and/or by means of vectors such as insects, mites, nematodes, fungi, dodders, pollen, seed, grafting, budding, vegetative propagation or soil (Sasaya 2015).

1.3 Bacterial Diseases

Bacterial diseases are the most devastating diseases of rice, found in tropical and temperate regions of the world, which include bacterial blight, leaf streak, foot rot, grain rot, sheath brown rot and pecky rice. Rice bacterial leaf blight (BLB), caused by *X. oryzae* pv. *oryzae* (*Xoo*), a Gram-negative bacterium, is one among the severely damaging diseases in rice (Ishiyama 1922). The outbreak of BLB as a seed-borne disease was first reported in 1884 at Japan (Saha et al. 2015). All the phases of growth are negatively impacted by BLB infection under favourable environmental conditions. However, rainy season and fast winds exaggerate the epidemic of BLB and result in further damage. The decrease in production caused by BLB range from 20 to 30% and can reach up to 80% in some of the cultivated area under severe infection (Chattopadhyay et al. 2017). Symptoms include yellow or white stripes on leaf blades, grayish leaves, wilting and stunted growth and plant death (Agrios 2005). The production of rice varieties with enhanced tolerance to bacterial disease is the efficient and sustainable approach for the management of disease, even though detection and subsequent selection of resistant source through screening under high pressure of BLB have been effectively exploited for the creation and release of resistant varieties. However, the co-evolution of new virulent mutant strains of *X. oryzae* pv. *oryzae* has always been a challenge for BLB resistance rice breeders. The recent advancements in the modern breeding approaches such as genomics, MAS and transgenics new genes that govern the resistance to bacterial blight have been identified, characterised, cloned and transferred to improve resistance into rice breeding. Compared to single gene introgression, pyramiding of multiple genes via MAS strategy has proven effective for disease management. Nonetheless, some of the advanced transgenic approaches such as overexpression, silencing and knockout of genes, genome editing techniques like TALEN (transcription activator like effector nucleus) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein) are also being employed in the recent past to develop complete resistance against this highly damaging bacterial disease (Mishra et al. 2018).

1.4 Fungal Diseases

Several species of fungi infect most important agricultural crops including rice and cause a significant reduction in overall production. Fungal diseases are considered as primary biotic stress that contributes to huge loss in rice yield (Srivastava et al. 2017). Agrios (2005) reported that about 70% of all major crop diseases are caused by fungi. The severely damaging fungal diseases of rice reported till now are "blast", "heliminthosporiose", "stem rot" and "foot rot", of these "blast" disease is more devastating and prevalent. Among the diseases of rice, false smut (FS) caused by *U. virens* decreases yield to a great extent. Recently *U. virens* has been placed in Clavicipitaceae and renamed as *Villosiclava virens* (Teleomorph) (Kepler et al. 2012; Tanaka et al. 2008), based on its ability to reproduce by both sexual and asexual means (Fu et al. 2012; Singh and Dubey 1984). The increased progress of FS in rice-growing area has been attributed to the use of nitrogen fertilisers and cultivation of hybrids on larger-scale cultivars (Deng 1989). Another ascomycete fungus *Magnaporthe oryzae* that causes a severe disease called as rice blast is the widespread in all rice-growing nations and led to 60–100% reduction in yield (Kihoro et al. 2013; Zhang et al. 2014).

1.5 Nematode Diseases

Plant–parasitic nematodes devastate the crops worldwide and pose a serious threat to the overall crop production (Raina et al. 2019a; Raina and Danish 2018). Among the biotic stresses, plant–parasitic nematodes represent another severe threat to the rice production (Soriano et al. 1999). As per Bridge et al. (2005), plant parasitic nematodes cause 10–25% yield losses annually worldwide, and economic loss corresponds to a monetary value of US$16 billion. Plant–parasitic nematodes attacks roots of herbs, shrubs and trees and upon infection reach to the aerial shoots and can feed on internal tissues (Soriano et al. 2004). Till now 150 species of plant–parasitic nematodes are known that can cause severe reduction in overall yield of rice due to very effective dispersal means viz. wind, water, animals and infected plant propagules. Among the different plant–parasitic nematodes, *Meloidogyne* spp. belong to a group of root-knot nematodes (RKNs), associated with root of crops, and induce gall formation in rice roots (De Waele and Elsen 2007), represented by more than 90 species (Moens et al. 2009). This RKN species is an obligate sedentary endoparasite that settles in roots and completes their entire life cycle inside the root cells and causes extensive damage to growth and development of rice (Williamson and Gleason 2003). The reduction in production increases when the soil is alternating dry and flooded under rain-fed conditions; therefore, water management practice influences the progress of disease (Prot and Matias 1995; Tandingan et al. 1996). In India, the first reports of RKN *M. graminicola* infecting rice were reported from Orissa (Patnaik 1969) and were equally prevalent on upland or lowland rice regions. In India, *M. graminicola* is widespread, and one of the dreadful nematode as is evident by its outbreak that devastated about 1500 ha cultivated land in Karnataka (Prasad and Varaprasad 2001).

1.6 Insect-borne Diseases

Among various obstacles in achieving the desired goals of rice production, insects incur about 30–40% of production loss. The agro-climatic conditions favourable for rice production are also conducive for rapid multiplication of insect pests (Heinrichs 1994). Infestation by insects, particularly stem borer, planthopper, leafhopper, gandhi bug, gall midge, rice leaffolder, rice hispa, cut worms and army worms is a serious challenge to achieve the desired goals of rice production (Pathak and Dyck 1973; Lou et al. 2013). However, the major insects that cause substantial reduction in rice yield include planthopper and leafhopper which cause direct damage and facilitate rapid transmission of viral diseases (Heinrichs 1994). About 100 insect pest species infest and damage the rice plant, among them 20 insect pests represent a serious threat to the production (Heinrichs 1994). The main stem borer species attacking every stage of growth include *Scirpophaga incertulas* (yellow stem borer) and *Sesamia inferens* (pink stem borer) and *Chilo polychrysus* in rice lands of Asia (Banerjee 1971; Pathak and Khan 1994). The degree of borer-caused reduction in rice yield has been estimated to range from 2 to 20% in non-outbreak per year and 30 to 70% in outbreak per year in India (Chelliah et al. 1989; Satpathi et al. 2012) and in Bangladesh (Catling et al. 1987), respectively. The estimated worldwide losses in rice production due to insect damage have been reported as 34.4% (Cramer 1967). Brown plant hopper (BPH) is also considered as the most serious damaging pests to the rice crop globally as they cause direct damage and also act as vectors for several dreadful viruses especially in rice lands with heavily fertilised soils. Chemical fertilisers and insecticide have been implemented to control the propagation of insect pests, but the limitation is the deterioration of grain quality. Hence, it is imperative to create rice cultivars with improved resistance to the insect pests. At IRRI researchers reported that rice fields protected from insects yielded almost double than unprotected rice fields and showed the impact of the insect pests on the overall rice production (Heinrichs 1994). Rice breeding programs have gained much success in the selection for insect-resistant rice varieties which showed less effect of borers' on the overall production (Khan et al. 2005). However, complete resistance against the YSB in cultivated rice varieties is still lacking and had also delayed the creation of resistant rice varieties (Bentur 2006). With the advancement in modern technology, breeding of insect pest stress tolerance have been improved by the identification, isolation and characterisation of genes that confer resistance to insect pest stresses. These genes can be introduced in rice varieties with higher yield but sensitive to insect borne diseases. The advancement in rice transgenic technologies has paved a way for the development of genetically modified (GM) rice that showed increased tolerance to insect pests (Bhattacharya et al. 2006).

2 Modern Breeding Approaches to Combat Biotic Stresses

The conventional breeding approaches have proven inefficient in improving the tolerance to biotic stress factors. To overcome the limitations of conventional breeding strategies, modern breeding approaches, viz. mutagenesis, transgenics and genomics, are employed for the creation of varieties with enhanced resistance to biotic stresses (Shamim and Singh 2017). At present, a collaborative research is going on to identify multiple stress factors involved in biotic stress tolerance and is discussed in detail in the following subsections.

2.1 Mutagenesis

In mutagenesis different chemical mutagens such as ethyl methane sulphonate, methyl methane sulphonate, sodium azide, hydrazine hydrates and physical mutagens such as gamma rays, X-rays, UV rays, heavy ion beams and laser beams are used by plant breeders to create rice genotypes with increased yield and better tolerance to biotic stresses (Raina et al. 2016; Khursheed et al. 2019). Among different breeding approaches, mutagenesis has proven to be a very effective tool for enhancing the genetic variation and improving resistance to biotic stresses. Additionally, mutagenesis equips the plant breeders to make the efficient selection of the desired genotype (Raina et al. 2018a Goyal et al. 2020). De Vries (1901) has first conceptualised the use of mutations for developing novel varieties in crops. Later on Stadler (1928) while working on barley has documented the practical significance of mutation breeding. Muller (1927) and Stadler (1928) and Ganger and Blakeslee (1927) were pioneers in authentication of use of electromagnetic waves in increasing the frequency of mutations in *Drosophila*, *Zea mays* and *Datura*, respectively. The first mutant Chlorina, mutant of *Nicotiana tabacum* was developed through the X-ray irradiation of floral buds in the 1930 (Coolhaas 1952). The collaborative research of FAO/IAEA lead to extensive and systematic research on use of mutations for the improvement of traits in wide range of crops. Several workers have employed mutation breeding for the improvement of different traits in different crops (Khursheed et al. 2015; Amin et al. 2016; Kalapchieva and Tomlekova 2016; Raina et al. 2019) that considerably reported the efficacy of induced mutations in crop improvement. Several researchers have employed different mutagens in different doses for creating varieties with desired traits in crops like lentil (Laskar et al. 2018a, b), cowpea (Raina et al. 2018b, 2020), mungbean (Wani et al. 2017), urdbean (Goyal et al. 2019a, b), fenugreek (Hasan et al. 2018), chickpea (Laskar et al. 2015; Raina et al. 2017, 2019b), black cumin (Amin et al. 2016, 2019; Tantray et al. 2017) and faba bean (Khursheed et al. 2018a, b, c). The plant traits improved by mutation breeding include yield, earliness, adaptability and tolerance to viral, bacterial, fungal and insect pests attack (Aetsveit et al. 1997; Khursheed et al. 2015, 2016; Laskar et al. 2019).

Mutation breeding in rice has been successful in developing and officially releasing 130 rice mutant varieties with improved traits like high yield, better grain quality and better resistance to biotic stresses. Earlier, the identification of mutated genes in subsequent generations was not possible due to lack of sophisticated biotechnological tools. In the recent years, a huge advancement in the modern breeding tools has led to the easy identification, isolation and transfer of newly mutated genes into stress susceptible variety without modifying the whole genome (Shu 2009). The possibilities of mutagenesis include development of new alleles and their incorporation into the new varieties that can be later on released as a commercial variety. Several attempts have been made to enhance tolerance to biotic stress in many crops including rice through mutagenesis. Mutant lines such as Camago-8, Heiseimochi, ITA 235, Shengba-simiao, Zhe 101, Zhengguang 1 and Zhongzao 21 have been developed that showed resistance to various viral diseases (Table 1) (mvd.iaea.org accessed July, 2019). Mutagenesis has also led to the development of 25 rice mutants that showed better resistance to bacterial diseases (Table 1) (mvd.iaea.org accessed July, 2019). Similarly mutation breeding has been successful in developing 100 mutant varieties of rice with enhanced tolerance to fungal diseases (Table 1) (mvd.iaea.org accessed July, 2019). The rice mutant variety named as RD6 has been developed by irradiating the non-glutinous variety Khao Dawk Mali 105 (KDML 105). The mutant variety showed promising results in terms of resistance to blast (*P. oryzae*) (Khambanonda 1978). The EMS dose of 0.1 and 0.2% concentrations was employed to develop blast resistance in the rice variety HYV Ratna (IR8/TKm 6). Few mutant lines in the M2–M5 generations showed better

Table 1 Role of mutagenesis in improving tolerance of rice to biotic stresses

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Zhengguang 1	China	1978	Gamma rays (300 Gy)	Yellow stunt virus
Camago-8	Costa Rica	1996	Gamma rays (250 Gy)	Resistance to blast and resistance to viruses
Heiseimochi	Japan	1988	Gamma rays (250 Gy)	Resistance to rice stripe virus
ITA 235	Nigeria	1988	Chemical mutagen	Semi-dwarfness and resistance to viruses (RYMV)
Zhongzao 21	China	2003	NA	Large spike and more grains, blast resistance
Shengba-simiao	China	2005	NA	Resistance to viruses
Zhe 101	China	2005	NA	Late maturity, high yield, resistance to blast and bacterial blight
Fulianai	China	1966	Gamma rays (200 Gy)	Short culm, resistance to blast, early maturity and high yield
Yangfuxian 2	China	1991	Gamma rays (300 Gy)	Resistance to bacterial diseases, high grain yield and good quality
Fuchuerai	China	1978	Gamma rays (350 Gy)	Shorter culm and improved resistance to bacterial leaf blight
Fuxian 6	China	1989	Hybridisation with mutant Fu 774	Early maturity (107–110 days), earlier and higher yielding, good resistance to BLB
Zhe 852	China	1989	Gamma rays (200 Gy)	Resistance to bacterial diseases, stress resistance, good grain quality characteristics and high grain yield
Zhefu 9	China	1990	It was developed by direct treatment with mutagen (IR50/44-1086)	Resistance to bacterial diseases, high yield
Yangfuxian 3	China	1993	Gamma rays (300 Gy)	High yield and resistance to bacterial diseases
Xiangzaoxian 21	China	1996	Combined treatment with gamma rays (288 Gy) and He-Ne laser	High yield, early maturity and resistance to bacterial diseases
Yuanjing 7	China	1999	Gamma rays (300 Gy)	High grain yield and resistance to bacterial diseases

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Xiangzaoxian 25	China	1997	Developed by hybridisation with one mutant Fu 26	High grain yield and resistance to bacterial diseases
Atomita 3	Indonesia	1990	Gamma rays (200 Gy)	Tolerance to brown plant hopper, BLB and bacterial leaf stripe, high yield
DB 250	Vietnam	1987	Gamma rays (250 Gy) and with 0.020% MNH during 6 hours	Resistance to lodging, resistance to bacterial blight and *Pyricularia oryzae* and yield (4.5 t/ha)
DT-10	Viet Nam	1989	Gamma rays (200 Gy) and with 0.025% MNH	Resistance to bacterial leaf blight and insects
Yangfuxian 9850	China	2004	Gamma rays (300 Gy)	High yield, good resistance to bacterial leaf blight, blast, sheath blight light
Yangfujing 4298	China	2004	NA	Improved agronomic traits and resistance to bacterial diseases
Yangfujing 4901	China	2004	Gamma rays	Strong resistance to blast, bacterial leaf blight, lodging resistance
Zhenuo #3	China	2003	Gamma rays	High yield and tolerance to bacterial diseases
Chiyou S162	China	2005	Gamma rays (300 Gy)	Improved yield, resistance to blast and bacterial blight
Nanhua 11	China	1987	Carbon dioxide laser irradiation of callus	High yield, resistance to bacterial diseases
Yangfuxian 5	China	2000	Gamma rays	High quality, high yield, multiple resistance
Kahayan	Indonesia	2002	Gamma rays (200 Gy)	High yield, resistance to leaf blight and amylose content (19–20%)
Winongo	Indonesia	2002	Gamma rays (200 Gy)	High yield, resistance to leaf blight and amylose content (19–20%)
Diah Suci	Indonesia	2003	Gamma rays (200 Gy)	High yield, resistance to leaf blight and amylose content (19–20%)
Mira 1	Indonesia	2006	Gamma rays (200 Gy)	High yield and resistance to bacterial diseases

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Aifu 9	China	1966	Gamma rays (300 Gy)	Short culm, resistance to blast and higher yield
Fuwan 23	China	1978	Gamma rays (300 Gy)	Resistance to yellow stunt and *Xanthomonas*, bigger spike, large grain size
Fuxuan 3	China	1970	Gamma rays (300 Gy)	Good tillering and resistance to blast
Fuxuan 124	China	1972	Gamma rays (300 Gy)	Resistance to blast
Jinfu 1	China	1969	Gamma rays (300 Gy)	Early maturity and resistance to blast
Kefuhong 2	China	1981	Developed by hybridisation with mutant IR8	Early maturity and resistance to blast
Wanfu 33	China	1978	Gamma rays (300 Gy)	Early maturity, resistance to blast
Wangeng 257	China	1975	Gamma rays (300 Gy)	Tolerance to fertilisers, resistance to blast and higher yield
Xiangfudao	China	1976	Gamma rays (300 Gy)	Resistance to blast and *Xanthomonas*
Xiongyue 613	China	1965	Gamma rays (200 Gy)	Moderate resistance to blast, higher yield and good quality
Yifunuo 1	China	1973	Gamma rays (100 Gy)	Resistance to blast, bigger spike and higher grain number
Fulianzao 3	China	1968	Gamma rays (300 Gy)	Early maturity, resistance to disease and short culm
Fushe 410	China	1974	Gamma rays (300 Gy)	Intermediate resistance to blast
Fu 769	China	1976	Gamma rays (300 Gy)	Resistance to diseases and high yield
Fu 756	China	1975	Gamma rays (300 Gy)	Resistance to diseases, good taste
M 112	China	1981	Gamma rays (300 Gy)	Resistance to *Sogatella furcifera* and high yield
Wanhongfu	China	1980	Gamma rays (350 Gy)	Resistance to low temperature and resistance to diseases
Zhuqin 40	China	1978	Gamma rays (300 Gy)	Resistance to blast
240	China	1980	Gamma rays (300 Gy)	Early maturity and resistance to diseases
Fushenongken 58	China	1973	Gamma rays (300 Gy)	Resistance to fungal diseases and high grain yield

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Ejingnuo 6	China	1986	Gamma rays (350 Gy)	Resistance to blast and blight, good grain quality and higher grain yield
Erjiufeng	China	1982	Gamma rays (350 Gy)	Higher yield, early maturity and resistance to fungal diseases
Taifu 4	China	1979	Gamma rays (200 Gy) and colchicines	Resistance to diseases and low application of fertilisers
652	China	1979	Gamma rays (300 Gy)	Resistance to fungal diseases
Xiushui 48	China	1981	Developed by hybridisation with mutant Funong 709	Resistance to blast, tolerance to low temperature and high yield
Xianghu 24	China	1983	It was developed by hybridisation with mutant Funong 709 [(Funong 709 × Jingyin 154) × Funong 709]	Resistance to blast and blight and glutinous grain type
Ailiutiaohong	China	1989	Gamma rays	Dwarfness (88 cm), high yield, resistance to *Pyricularia oryzae*, resistance to insects
Qingwei 1	China	1985	Gamma rays	High yield, resistance to diseases and late maturity
Fu 8-1	China	1988	Gamma rays (350 Gy)	Resistance to fungal diseases and high grain yield
Tangernian	China	1985	Gamma rays	High yield, resistance to diseases and late maturity
Wanhua	China	1983	Gamma rays (350 Gy)	Semi-dwarfness, resistance to diseases, superior grain quality and high yield
Fuwan 81-548	China	1989	Gamma rays (300 Gy)	Good quality and resistance to fungal diseases
Meisanwu 2	China	1990	Gamma rays (150 Gy)	Resistance to fungal diseases and insects
Xiuxui 117	China	1984	It was developed by direct use of mutagen treatment on Funong 709/Zaison/Funong709/ Chengbaoxifeng	Resistance to fungal diseases and altered maturity

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Xianghu 93	China	1984	(Funong 709 × Jingyin 154) × Funong 709	Resistance to fungal diseases and altered maturity
Zijiangnuo	China	1984	(Fuhong 3 × Xinbasi × Nenjing 15 gamma)	Resistance to fungal diseases and high grain yield
Xiushui 04	China	1985	(Ze 21/Funong 709/Dan 209)	Resistance to fungal diseases, blast and bacterial blight, high yield
Ganwannuo	China	1993	Developed by hybridisation with one mutant MY82166	High grain yield and resistance to fungal diseases
Wandao 20	China	1994	Ion beams	Altered maturity and resistance to fungal diseases
Wandao 45	China	1994	Ion beams	Altered maturity and resistance to fungal diseases
Shenxiangjing	China	1994	NA	Improved plant structure and resistance to fungal diseases
Zhefu 762	China	1993	NA	High grain yield (5–10%), high resistance to blast, bacterial blight resistance
Ganwanxian 23	China	1994	It was developed by hybridisation with one mutant (TR 841 × M79215)	High quality and resistance to fungal diseases
Fuxuan 8	China	1998	(Fu 8329 × Fu 8105 × IR13471–74-1)	Resistance to fungal diseases
Camago-8	Costa Rica	1996	Gamma rays (250 Gy)	Resistance to blast and resistance to viruses
Camago-8	Costa Rica	1996	Gamma rays (250 Gy)	Resistance to blast and resistance to viruses
UNP 9027	Costa Rica	1994	Gamma rays (200 Gy)	Resistance to *Pyricularia oryzae*
IRAT 216	Cote D'Ivoire	1985	Gamma rays	Good adaptability to wetland rice culture, resistance to *Pyricularia*
Calendal	France	1979	Gamma rays	Longer grains, improved trashability, resistance to *Sclerotinium oryzae*

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Marathon	France	1985	Gamma rays	Resistance to *Pyricularia*
Nucleoryza	Hungary	1972	Fast neutrons (25 krad)	Early maturity, maintained blast resistance and improved yield
Mutashali	Hungary	1980	Fast neutrons (20 Gy)	Resistance to *Pyricularia oryzae* and high yield
Pusa-NR-381	India	1989	Gamma rays	Resistance to blast
CRM 49	India	1999	0.001 M sodium azide (NaN3)	Resistance to blast disease
CRM 51	India	1999	0.001 M sodium azide (NaN3)	Resistance to blast disease
CRM 53	India	1999	0.66% EMS	Resistance to blast disease
Atomita 1	Indonesia	1982	Gamma rays (200 Gy)	Early maturity, resistance to BPH, GLH and blast
Danau atas	Indonesia	1988	Gamma rays (400 Gy)	Resistance to blast, high yield
Fulgente	Italy	1973	X-rays (250 Gy)	Blast resistance and high productivity
Sachiminori	Japan	1978	Gamma rays	Stiff culm and resistance to blast
ITA 123	Nigeria	1980	Gamma rays (20–2000 Gy)	Semi-dwarfness and resistance to rice blast
RD 6	Thailand	1977	Gamma rays (200 Gy)	Glutinous endosperm and improved resistance to blast
Pooya	Iran, Islamic Republic of	2004	Gamma rays (150 Gy)	Resistance to lodging, resistance to blast and higher yield
Tabesh	Iran, Islamic Republic of	2004	Gamma rays (150 Gy)	Resistance to lodging, short culm, tolerance to blast and higher yield
Minnuo 706	China	1988	Gamma rays (250 Gy)	Good tillering, higher yield, glutinous, resistance to blast, good quality
Jinhang-simiao	China	2006	Aerospace	Resistance to fungal diseases and good quality
Huahang-simiao	China	2006	Aerospace	Resistance to fungal diseases and good quality

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Peiza 130	China	2008	Aerospace	High yield, resistance to fungal diseases and early maturity
Liangyouhang 2	China	2008	Aerospace	High yield, resistance to fungal diseases, blast and bacterial blight and good grain quality
Hangxiang 18	China	2008	Aerospace	Late maturity and resistance to fungal diseases
Yuanjing 41	China	2004	NA	Resistance to fungal diseases
Zhenuo 5	China	2004	NA	Resistance to fungal diseases
Yuanjing 35	China	2005	NA	Resistance to fungal diseases
Guangyinruanzhan	China	2008	Physical mutagen	High yield, high quality, resistance to blast and bacterial leaf blight
Early Samba	India	2000	It is a mutant from BPT-5204	Dwarfness, white MS grains, tolerance to SB, yield (60–65 Q/ha)
IACuba 28	Cuba	2001	Fast neutrons (20 Gy)	Large grain size, high yield, resistance to blast
Michinoku-wase	Japan	1988	Gamma rays (200 Gy)	Resistance to leaf blast
Okini-iri	Japan	1996	Gamma rays (200 Gy)	Superior eating quality and high field resistance to blast
Hayatsukushi	Japan	1997	Gamma rays (200 Gy)	Extremely early-maturity and highest field resistance to blast
Hiroshima No. 21	Japan	1998	Gamma rays (200 Gy)	Resistance to leaf and panicle blast
Koshihikari Toyama BL No. 2	Japan	1998	Gamma rays	Resistance to fungal diseases
Aichi-no-kaori SBL	Japan	1999	Gamma rays (200 Gy)	High resistance to rice stripe disease and panicle blast and BLB
Fusa-no-mai	Japan	2000	Gamma rays (200 Gy)	Suitable for sake brewing, high cold resistance, high resistance to the panicle blast
Koshihikari Niigata BL No. 4	Japan	2002	Gamma rays (200 Gy)	Resistance to blast
Koimusubi	Japan	2002	Gamma rays (200 Gy)	Excellent cultivation characteristics, blast resistance

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Improved trait (s)
Churahikari	Japan	2003	Gamma rays (200 Gy)	High resistance to blast, medium-late maturity, shorter culm
Sai-no-kagayaki	Japan	2002	Gamma rays (200 Gy)	Field resistance to blast and stripe disease and green rice leaf hopper
Zhejing 41	China	2009	NA	Resistance to blast, bacterial leaf blight and brown plant hopper
Moretsu	Japan		Chemical mutagen MNU	High resistance to lodging and high resistance to stripe rust
M 114	China	1981	Gamma rays	Tolerance to low temperature and resistance to Fulgorid plant hopper
Meisanwu 2	China	1990	Gamma rays (150 Gy)	Resistance to fungal diseases and resistance to insects
Pusa-NR-555-5	India	1990	Gamma rays	Resistance to pests and resistance to diseases
Pusa-NR-570-17	India	1990	Gamma rays	Resistance to pests and resistance to diseases
Pusa-NR-519	India	1990	Gamma rays	Resistance to pests and resistance to diseases
Atomita 3	Indonesia	1990	Gamma rays (200 Gy)	Resistance to brown plant hopper resistance to BLB, bacterial leaf stripe, high yield
VN24-4	Viet Nam	2009	Developed by hybridisation with female variety IR64 and male mutant variety VND95-19	Bigger panicles, stiff culms, strongly seedling vigour, high tolerance to pest and diseases (BPH & GSV)

Source: mvd.iaea.org (MVD-2019)

resistance to blast disease (Kaur et al. 1971). Attempt to develop blast resistance via the use of 100 Gy gamma rays in the F1 progeny lead to the isolation of mutant R917 with improved resistance (Zhang et al. 2003). Similarly, the Mtu 17 blast-resistant mutants with elite agronomic traits were developed through chemomutagenesis with diethyl sulphate (dES) (Gangadharan and Mathur 1976). Mohamad et al. (2006) and Azlan et al. (2004) have reported several blast-resistant mutant lines, such as Mahsuri Mutant SPM 129, SPM 130 and SPM 142, which have been developed in Malaysia. Another mutant variety "Zhefu 802" with high resistance to rice blast has been developed through gamma irradiation of variety "Simei No. 2"

(Ahloowalia et al. 2004; Shu et al. 1997). Mutagenesis has also been successful in the development of rice mutant varieties which showed enhanced resistance to the insect pest attack. For instance, the varieties such as Atomita 3, M 114, Meisanwu 2, Pusa-NR-519, Pusa-NR-555-5, Pusa-NR-570-17 and VN24-4 have been developed to mitigate the effect of insect pest attack (Table 1) (mvd.iaea.org accessed July, 2019). However, mutagenesis is under progress to develop rice varieties with improved resistance to nematodes, and till date no variety of rice with tolerance to nematode attack has been developed.

2.2 Transgenics

Many morpho-physiological and biochemical traits linked with disease resistance are governed by different sets of genes. Molecular breeding have been employed for the creation of varieties with improved resistance by insertion of new resistance genes into promising lines. Conventional breeding approaches such as selection and hybridisation have resulted in the development of new varieties that can persist under pest and pathogen attack, but these approaches are cumbersome and require long duration of time. This necessitates the implementation of new and effective strategies for disease management and development of varieties with enhanced resistance to wide range of biotic stresses (Collard and Mackill 2008; Hasan et al. 2015). Modern biotechnological tools have proven very effective in enhancing the yield and reducing the crop loss due to single and/or multiple biotic stresses (Onaga and Wydra 2016). The advent of transgenics and single-gene approach where stress-responsive genes are overexpressed in stress-sensitive plants, have paved a way for the quick and efficient development of cultivars with improved tolerance to biotic stresses. Even though insecticides have been effective in controlling the viral disease, the high prices of insecticide and its environment hazard are the main demerits. Hence, transgenics wherein genes that confer tolerance to stress are introduced and overexpressed in stress-susceptible varieties have proven effective in curbing the virus infestation. Sasaya et al. (2013) developed transgenic rice with improved resistance against two tenuiviruses by introduction of double-stranded RNA. The results showed increased resistance to *rice stripe virus* (RSV) and *rice dwarf virus* (RDV) infection in transgenic rice plants induced by different RNAi-targets of RSV and RDV genes (Table 2). Another dreadful viral diseases of rice is caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) with the help of a vector *Nephotettix virescens* (green leafhopper) that facilitates its quick transmission from infected to non-infected plants. At present efforts are being made by employing coat protein-mediated resistance strategy wherein rice plants have been transformed by the insertion of RTSV replicase gene. The results revealed that transformed rice were more resistant to RTSV and also showed improvement in yield (Huet et al. 1999).

Song et al. (1995) developed transgenic rice by the insertion of *Xa21* gene. Transgenic rice plants with *Xa21* revealed enhanced tolerance to bacterial blight and manifold increase in yield and yield attributed traits due to least damage caused

Table 2 Role of transgenics in improving the resistance of rice crop against biotic stresses

Gene(s)	Trait	References
cry1Ac and *CpT1*	Insect resistance	Han et al. (2006)
cry1Ab	Insect resistance	Wang et al. (2014)
Xa21	Bacterial blight resistance	Tu J et al. (2000a)
Bar	Sheath blight disease	Uchimiya et al. (1993)
Chi 11	Sheath blight disease	Lin et al. (1995)
TLP-D34	Sheath blight disease	Datta et al. (1999)
RC 7	Sheath blight disease	Datta et al. (2000, 2001)
pinA, pinB	Sheath blight disease	Krishnamurthy et al. (2001)
Chi, Xa21, Bt	Sheath blight disease	Datta et al. (2002)
ChiC	Fungal disease resistance	Itoh et al. (2003)
Gns1	Fungal disease resistance	Nishizawa et al. (2003)
Ech42, nag70, gluc78	Fungal disease resistance	Liu et al. (2004)
OsNPR1	Bacterial disease resistance	Yuan et al. (2007)
AtNPR1	Fungal and bacterial disease resistance	Quilis et al. (2008)
Cht42	Fungal disease resistance	Shah et al. (2009)
Pi-d2	Fungal disease resistance	Chen et al. (2011)
OsMPK6	Bacterial disease resistance	Shen et al. (2010)
Xa3/Xa26	Bacterial disease resistance	Li et al. (2012)
HPL3	Bacterial disease resistance	Tong et al. (2012)
ACS2	Fungal disease resistance	Helliwell et al. (2013)
OsGA20ox3	Fungal and bacterial disease resistance	Qin et al. (2013)
RTBV coat protein	Viral disease resistance	Ganesan et al. (2009)
RSTV RNA	Viral disease resistance	Verma et al. (2012)
PINII-2X	Insect resistance	Bu et al. (2006)
ASAL	Insect resistance	Bharathi et al. (2008)
ASAL, GNA	Insect resistance	Bharathi et al. (2011)
DB1	Insect resistance	Yoshimura et al. (2012)
cry1Ab	Insect resistance	Shu et al. (2000)
cry2A	Insect resistance	Chen et al. (2005)

by the pathogen under field conditions (Tu J et al. 2000a). Wang et al. (2017) developed transgenic rice cultivar Nipponbare by the introduction of Xa10-like genes. The Xa10-like gene encodes for AvrXa10 (transcription activator-like effector) which binds to Xa-10 and activates its expression. Upon subsequent infection by *Xanthomonas oryzae* pv. *oryzae*, transgenic rice cultivar revealed improved tolerance to bacterial blight and showed higher yield.

Cao et al. (2007) reported a disease-resistant (*R*) multigene family comprising of *Xa3*/*Xa26*, *MRKa*, *MRKc* and *MRKd* encoding a leucine-rich repeat (LRR) receptor kinase-type protein in rice cultivars that govern tolerance to *Xanthomonas oryzae* pv. *oryzae*. Their results revealed few R genes under strong constitutive promoter conferred tolerance to *Xanthomonas oryzae* pv. *oryzae* as compared to their native promoters. Rice plants harbouring another gene *Xa26*, isolated from rice, also revealed high level of resistance against bacterial blight (Sun et al. 2004).

Genetically engineered rice plants expressing AP1 (ferredoxin-like protein) isolated from sweet pepper showed improved resistance to *X. oryzae* (Tang et al. 2001). This confirms the ap1 gene could be used to induce bacterial resistance in disease-susceptible rice cultivars (Table 2).

Several genes that confer resistance against several fungal diseases were identified and subsequently employed in genetic engineering programs to improve tolerance to fungal attack in disease-susceptible rice cultivars. Dai et al. (2010) have recently reported a cloned *Pi-ta* gene that confers substantial tolerance against rice blast caused by a pathogenic fungi *Magnaporthe grisea*. Liu et al. (2009) have mapped the loci that govern tolerance to sheath blight caused by a pathogenic fungi *Rhizoctonia solani*. They were successful in identifying several molecular markers associated with sheath blight resistance by means of crossing between resistant transgenic and sensitive non-transgenic rice cultivars (Table 2). Datta et al. (2003) reported a gene *PR-3* that confers tolerance to sheath blight and hence can be used in transgenics for the improvement of fungal disease resistance. Transgenic rice harbouring *Rir1b* gene (defence-related gene) isolated from cereals reflected improved resistance to rice blast (Mauch et al. 1998; Li et al. 2009). Different proteins/genes isolated from different organisms have been recognised as potential source to confer resistance against several fungi species in rice plants (Kumar et al. 2018). For instance, transgenic rice harbouring and co-expressing *ap24* (tobacco osmotin), *chi11* (rice chitinase) (Sripriya et al. 2017) and chitinase and oxalate oxidase 4 (Karmakar et al. 2016) and overexpression of *LOC_Os11g47510* chitinase gene showed more resistant to sheath blight disease (Richa et al. 2017). Several proteins such as puroindoline proteins (Krishnamurthy et al. 2001), flavonoid pathway genes (Gandikota et al. 2001), trichosanthins (Yuan et al. 2002), defensins (Kanzaki et al. 2002), phytoalexins (Hasegawa et al. 2010) and antifungal protein from *Aspergillus flavus* (Coca et al. 2004) are known to play a vital role in combating the fungal diseases and can be promising candidates in transgenics.

Transgenics have also been very successful in developing insect-resistant crops including rice (Brooks and Barfoot 2013). Fujimoto et al. (1993) have reported the development of insect-resistant rice plants about two decades ago. Transgenic rice harbouring *cry* genes isolated from *Bacillus thuringiensis* are currently under field trials, the preliminary results reveal a substantial resistance against stem borers and leaffolders (Cohen et al. 2008; Wang et al. 2014). Similarly, High et al. (2004) have reported that Bt rice showed significant resistance against lepidopterous pests in Asia. Transgenic rice carrying a synthetic c*ry1Ab* gene reflected substantial tolerance to several lepidopterous pests of rice (Shu et al. 2000). Moreover, field studies led to the identification of two lines from Bt rice plants that showed complete resistance to lepidopteran pests (Kumar et al. 2008; Wang et al. 2014). In China hybrid rice plants with improved tolerance to rice leaffolder and yellow stem borer were developed (Tu JM et al. 2000b; Chen et al. 2011). In Pakistan and Mediterranean region, insect-resistant Bt rice have been developed (Breitler et al. 2004) which reflected complete resistance against target yellow stem borer and rice leaffolder. Studies are being carried out to pyramid *cry1Ab* or *cry1Ac* with either *cry2A* or *cry9C* for high resistance in Bt rice (Alcantara et al. 2004; Ansari et al. 2015). In addition to *cry* genes, *gna* lectin gene isolated from snowdrop (*Galanthus nivalis*) induced higher levels of tolerance against several pests (Ramesh et al. 2004). The transgenic rice harbouring protease inhibitors and lectins showed improved tolerance against insect pests, and hence they may also serve as potential source to develop rice with improved resistance against several insects (Kumar et al. 2008) (Table 2).

3 Genomics

Biotic stress incurs a substantial decrease in the average annual yield of rice in rice fields worldwide (Heinrichs and Muniappan 2017). In the current scenario of climate change and evolution of pests and pathogens, plants face biotic stress at rapid pace (Cohen and Leach 2019). Conventional breeding strategies practiced thousands of years have resulted into varieties that were much tolerant to disease outbreaks (Buddenhagen 1983). However, the co-evolution of new virulent strains on a much faster pace further posed challenges before plant breeders and geneticists. The conventional breeding approaches are cumbersome, laborious and requires a long duration of time to improve a trait and all these drawbacks have led to the rise of marker-assisted breeding for developing varieties with improved tolerance to diseases. Initially, molecular markers like RFLP, RAPD, AFLP, SSRs and SNPs have played a major role in marker-assisted breeding for developing varieties with increased resistance to a wide range of biotic stresses (Table 3). Later on mapping of quantitative trait loci provided more insights into the underlying mechanism of tolerance to viral, bacterial, fungal and insect pest attack in rice. A recently developed genome editing techniques have superseded the drawbacks of conventional breeding approaches and have paved a new way for crop improvement. Genome editing approaches have been used to modify various disease-related genes to enhance disease resistance in rice. In genome editing techniques, site-specific nucleases are employed to engineer genes of interest at desired loci in the genome. Transcription activator-like effectors (TALEs) from *Xanthomonas* species such as AvrXa7 and PthXo3 target and modify the sugar transporter SWEET gene and sucrose efflux transporter OsSWEET14 gene to facilitate the influx of sugars from the plant cell to the pathogen (Antony et al. 2010; Cohn et al. 2014) (Table 3). Transcription activator-like effector nucleases (TALEN) technology was used to modify the bacterial protein binding site on OsSWEET14 gene to impart resistance against *Xanthomonas* causing bacterial blight (Li et al. 2012). TALEN technology is effective in disrupting EBEtal7 binding site in promoter of Os09g29100 gene, which could significantly decrease bacterial blight (Cai et al. 2017). Li et al. (2012) reported that collaborative approach of targeted mutagenesis and TALEN technology was effective in disrupting the *Os11N3* gene susceptible for bacterial blight in rice. Recently, a simple robust and effective gene editing technology have been developed wherein the disease susceptible genes can be targeted and edited to improve disease resistance in rice. CRISPR/Cas9 have been used to target and edit by deleting nine and seven nucleotides from promoter of *OsSWEET14* and *OsSWEET11* genes,

Table 3 Role of genomics in improving the resistance of rice crop against biotic stresses

Gene (s)	Improved trait (s)	Reference
OsSWEET13	Enhanced resistance to bacterial blight	Li et al. (2012)
OsSWEET13	Enhanced resistance to bacterial blight	Zhou et al. (2015)
OsSWEET13	Enhanced resistance to bacterial blight	Blanvillain-Baufum et al. (2017)
Os09g29100	Enhanced resistance to bacterial leaf streak	Cai et al. (2017)
OsERF922 CRISPR/Cas9	Enhanced resistance to blast disease	Wang et al. (2016)
cry1Ab or *cry1Ac*	Yellow stem borer, stripe stem borer	Shu et al. (2000)
cry1Aa or *cry1Ab*	Stripe stem borer	Breitler et al. (2004)
cry1Ab and *cry1Ac*	Yellow stem borer	Ramesh et al. (2004)
cry1Ab	Stripe stem borer	Cotsaftis et al. (2002)
cry1Ab	Yellow stem borer, rice leaffolder	Bashir et al. (2005)
cry, *Xa21* and *RC7*	Yellow stem borer, bacterial blight, sheath blight	Datta et al. (2003)
gna and *cry1Ac*	Homopteran, coleopteran and lepidopteran insects	Nagadhara et al. (2003)
Itr1	Rice weevil	Alfonso-Rubi et al. (2003)
cry1Ac and *cry2A*	Yellow stem borer, rice leaffolder	Mahmood-ur-Rahman et al. (2007)
Bt and *CpTI*	Insect resistance	Rong et al. (2007)
Bt, protease inhibitors, enzymes, and plant lectins	Insect resistance	Deka and Barthakur 2010
cry2Aa	Insect resistance	Wang et al. (2012)
cry1Ab	Insect resistance	Wang et al. (2014)
xa5, *xa13* and *XA21*	Bacterial Blight resistance	Singh et al. (2001)
Xa39(t)	Bacterial	Sundaram et al. (2014)
Xa38, *xa13*, *XA21*	Blight resistance	Sundaram et al. (2014)
XA21, *xa13*, *xa5* and *Xa4*	Bacterial	Sundaram et al. (2014)
XA21, *xa13 and xa5*	Blight resistance	Sundaram et al. (2014)
XA21 and *xa13*	Bacterial	Sundaram et al. (2014)

thereby increasing bacterial leaf blight resistance in rice (Jiang et al. 2013). In indica rice, IR24 a null mutation in OsSWEET13 was created by means of CRISPR/Cas9 to avert its neutralisation by the TAL effector gene pthXo2, thereby increasing tolerance against bacterial blight disease (Zhou et al. 2015). Wang et al. (2016) reported the enhancement of tolerance against rice blast by targeting the *OsERF922* gene via a CRISPR/Cas9 technology. Another CRISPR/Cas9-mediated editing of eIF4G gene has led to an improvement in tolerance against rice tungro spherical virus RTSV (Macovei et al. 2018). This confirms that CRISPR/Cas9 is a coherent tool for improving resistance against almost all diseases in rice.

4 Conclusion

Biotic stresses that devastate the rice production include virus, bacteria, fungi, nematode and insect pests. Among different breeding approaches, mutagenesis have proven very effective tool for enhancing the genetic variation and improving resistance to biotic stresses. The recently developed genome editing techniques have superseded the drawbacks of conventional breeding approaches and have paved a new way for crop improvement. Genome editing approaches have been used to modify various disease-related genes to enhance disease resistance in rice. Overall, the modern biotechnological tools such as mutagenesis, transgenics and genomics have led to the identification, cloning and characterisation of genes (from different organism) followed by its insertion into the rice plants with the aim of decreasing the yield loss incurred by the different biotic stresses.

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