



GENETIC ENGINEERING FOR BIOTIC STRESS MANAGEMENT IN RICE

Hamidullayev F.L.

Tashkent State Technical University named after Islam Karimov
hamidullayevfatxulla@gmail.com

Kurbanov G.A.

National University of Uzbekistan named after Mirzo Ulugbek
 Military training center p-pk
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Abstract Rice yield is affected by various biotic stresses including fungi, bacteria, viruses, parasites, nematodes, weeds and insects, posing a major threat to global food security. Therefore, one of the major objectives of rice breeders is to develop rice cultivars resistant to biotic stresses and it has been achieved to large extent through traditional and molecular breeding approaches. However, frequent breakdown of resistance to these biotic stresses is a challenging issue and therefore, continuous efforts are needed to develop cultivars with durable resistance. Recently, genetic engineering technologies like transgenics and RNA-i have enabled breeders to develop such durable resistance in rice against number of bacterial, fungal and viral diseases by utilizing the genes conferring resistance to trait and isolated from various organisms like plants, animals, microbes, etc. Genetic engineering is more preferred in some of the cases as it has advantages like requirement of lesser duration, no linkage drag and no crossing barrier compared to molecular breeding. Although varieties developed through genetic engineering require prior regulation before commercialization, it has the enormous potential to develop plants resistant/tolerant to biotic stresses. Present status of use of genetic engineering for developing biotic stress resistance in rice is briefly described in this chapter.

Keywords Biotic stress, Genetic engineering, Rice, Transgenic, Disease, Insects

Introduction

Rice (*Oryza sativa* L.) is one of the most abundant and widely grown crops in the world. Rice cultivation is allied to human progress and it has played a significant role in human nutrition and culture for the past 100 centuries. Rice is an important staple food crop and needs continuous improvement to fulfil the growing demand of the continuously rising population. With the advent of an increase in several environmental stresses, rice production has been severely hampered. Rice breeding programs are highly aided by standardized molecular biology tools in rice along with the availability of fine genetic maps and transformation and regeneration protocols. Rice is considered a model organism for cereal crops because of its extensive similarity with other family members of Poaceae. *Oryza* is a genus belonging to the tribe Oryzae of a Poaceae family. The taxonomy of rice has been characterized as Kingdom: Plants, Subkingdom: Vascular plants, Superdivision: Seed plants, Division: Flowering plants, Class: Monocotyledons, Subclass: Commelinidae, Order: Cyperales, Family: Poaceae, Genus: *Oryza* L. The genus *Oryza* consists of 22 distinct species broadly grouped into four complexes viz., *sativa*, *officinalis*, *ridley*, and *meyeriana*. Among these species, only *Oryza sativa* and *Oryza glaberrima* are widely cultivated. There are twenty-one wild species of genus *Oryza*, and ten recognized genome types in rice namely, AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, HHKK. In

the past decade, rice has been a model for comparative genomics of plants as well as for cereal genomics and broad molecular biology studies. Many factors are responsible for declaring it as a model plant. This economically important cereal remains not merely an academic pursuit but established itself to find future prospects. Recent advancements in biotechnology could be used for improvement in rice production by unlocking the genetic and physiological mechanism of the rice plant to expand its geographical adaptation and to overcome various biotic and abiotic constraints. Rice breeding programs are mainly focused on improving the nutrition, quality and yield of grains along with several other important agronomic traits like early maturity, resistance to various environmental stresses, nutrient use efficiency, resistance to lodging and shattering, etc. In this chapter, we are mainly focusing on the biotechnological efforts to improve rice crops against various biotic stresses.

Biotic Stresses in Rice

Plants are often encountered with several other organisms in their whole life cycle and these organisms such as fungi, bacteria, viruses, parasites, nematodes, weeds, insects, etc. cause significant damages to plant yield. Biotic stress affects overall growth and development of plants, quantity and quality of seeds, and ultimately it affects yield. Among the biotic stresses of rice, diseases and insect pests are a serious threat to production and productivity. The International Rice Research Institute (IRRI), Philippines, estimates that in order to feed the increasing global population, rice production must escalate by one third by the year 2020 shows that there is a requirement of 25% more rice by 2030 for ensuring food security. To ensure global food security, sustainable rice production is very important and therefore, it is essential to develop rice cultivars that are resistant or tolerant to biotic stresses. To encounter the challenges arising due to various biotic stresses and sustainable yield, rice-growing area needs to be enhanced, however, the increasing population resulting in increased urbanization is a major hurdle. On the other hand, it is necessary to develop and grow biotic stress resistance cultivars that can yield comparatively better in stress conditions. Breeding programs in rice for developing resistance against different biotic factors utilize several landraces and wild relatives as a source of resistance genes. The foremost aim of breeders or biotechnologists is to identify the genomic loci or genes responsible for conferring resistance to disease or pests. Such fully characterized genes can be further transferred to superior cultivars through traditional breeding where the natural crossing is possible. However, many times the gene pool lacks the potential genes that can be transferred through breeding. Under these conditions, the genes from other systems can be effectively utilized and transferred in superior cultivars by using genetic engineering to develop transgenic crops.

Genetic Engineering or Transgenic Technology

Since the beginning of genetic engineering era, this technology has been considered as one of the most potential technologies for crop improvement. It has the potential of increasing yield as well as minimizing crop losses due to several environmental stresses. Transgenic technology is successful in large number of crops and the products range from insect-resistant to herbicide-resistant, virus-resistant, better oil quality, drought-tolerant, etc. Transgenic crops are growing worldwide in more which shows its potential and wide acceptance. The prerequisite for any transgenic crop development is to have well-characterized genes and promoters responsible for governing resistance to particular biotic stress. A large number of genes playing a vital role in resistance to various biotic stresses has been cloned and characterized using modern genomics and molecular biology tools. Rice plants have been



successfully transformed by many of these significantly promising genes to make the plants stress-tolerant or disease resistant. Evaluation of these transgenic rice plants under greenhouses as well as field conditions is being carried out in different countries for biosafety analysis. Thus, transgenic technology is one of the most potential technologies for the rice improvement program.

Resistance to Bacterial Diseases

Bacterial diseases in rice are also serious challenges that severely impact rice yield. Bacterial blight is one of the most destructive diseases and severe threats to rice production and it is caused by *Xanthomonas oryzae* pv. *Oryzae*. To develop resistance against bacterial blight in rice, 10 R genes have been characterized so far. Some of the R genes have been successfully transferred from resistant line to susceptible line through marker-assisted backcross breeding. For example, xa5, xa13, and Xa21 genes from Swarna Bacterial Blight resistant pyramid line were transferred to elite deep-water Jalmagna cultivar. Pathogenesis-related (PR) genes are vital for disease resistance and their expression is mainly regulated by ethylene-responsive (ERF) transcription factors. Another important resistance providing gene is *Oryza cystatin1* (OC1) which is a protease inhibitor in rice seeds and has been transferred to various crops such as rice (Duan et al), wheat, oilseed rape and eggplant. In eukaryotic organisms, stress response is mediated by mitogen-activated protein kinase (MAPK) cascade. One such stress-responsive gene OsMAPK5 which is induced by abscisic acid, pathogen infection, and abiotic stresses have been cloned and characterized from rice. The characterization of this gene was done by over-expression by 35S promoter as well as by suppression using RNA interference. OsMAPK5 gene is a negative regulator of PR genes as RNAi transgenic lines constitutively expressed these PR genes. These transgenic plants exhibited significant resistance against bacterial (*Burkholderia glumae*) and fungal (*Magnaporthe grisea*) pathogens. However, there observed a negative impact on tolerance to drought, salt, and cold which was significantly reduced in these plants. Over-expression lines, in contrast, showed positive impact on these abiotic stresses due to increased kinase activity. Thus, OsMAPK5 is a positive regulator of abiotic stress tolerance and a negative regulator of disease resistance through modulating the expression of PR genes.

Resistance to Fungal Diseases

Among the various fungal diseases, blast disease caused by *Magnaporthe oryzae* is the most devastating, and, dwindle yield by as much as 75% in infected areas. The competence of *M. oryzae* to suppress plant immune system and ability to modify host metabolism and cell signalling is the key feature of its virulent lifestyle which makes it a model phytopathogen. It is a filamentous, heterothallic, ascomycete fungus and has the ability to grow away from the host plant in standard growth media. The fungus is extensively studied by classical genetics and it is easily and efficiently transformed with several selectable markers. Traditionally, the fungal diseases are controlled by low-cost remedial strategies such as use of uninfected seeds, low doses of nitrogen fertilizers, burning of plant remains after harvesting, etc. But these strategies are least effective in eliminating the infections once epidemic spreads at large-scale under field conditions. Although several integrated management practices like cultural, mechanical and chemical have been reported to manage the disease, the management through host resistance is considered as one of the best methods. Resistance is actually a result of incompatible interaction between host and the pathogen. However, complete resistance needs unified functioning of biochemical as well as genetic factors to stay protected against



pathogen. Rice crop season is highly favourable to Magnaporthe pathogen which is of highly variable nature and therefore, it is difficult to manage rice blast disease by the use of resistant cultivars. Due to these reasons, blast-resistant varieties do not sustain in the disease-prone areas and results in breakdown of resistance within only 2–3 years of their introduction. Though blast genetics study started in the early 1910s, Pib was the first gene cloned for blast resistance in 1999 by a group of Takuji Sasaki in Japan. In the next two decades, a total of twenty-four blast resistance genes have been mapped, cloned, and characterized in rice. Second gene to be cloned was Pi-ta, with simultaneous cloning and characterization of the corresponding Avr-Pi-ta gene from *M. Oryzae*. After a gap of five years, third gene Pi-kh (now designated as Pi54) was cloned and characterized from Indica rice line Tetep which is prevalent in Northwestern Himalayan region of India. This gene provides resistance to different strains of *M. Oryzae*. Other orthologs of Pi54 i.e. Pi54rh and Pi54of were also cloned and characterized showing broad-spectrum resistance against various isolates of *M. oryzae* (Das et al. Devanna et al). Till date, more than 100 R genes have been identified and amongst them, 24 are cloned. The OsNAC6 gene which belongs to NAC transcription factor family is upregulated by wounding, blast disease and abiotic stresses such as cold drought and salt. Rice plants transformed with OsNAC6 are significantly tolerant to dehydration and high salt along with resistance to blast disease. However, these transgenic plants showed growth retardation and a severe impact on reproductive yields. Thus, transcription factor OsNAC6 is a potential transcriptional activator of genes governing biotic and abiotic stress response in plants. Secondary metabolites like phytoalexins are also one of the targets for enhancing the resistance against diseases in plants. Phytoalexins play a very important role in defense mechanisms in many plant species. To increase the synthesis of phytoalexin in grapes, plants were transformed with stilbene synthase gene (STS) of Vst1 which is a key enzyme for phytoalexin synthesis. The plants showed significant resistance to *Pyricularia oryzae*. Phytoalexin synthesis also occurs in rice in response to UV and blast infection and its synthesis is regulated by MAPK cascade, especially OsMAPKK6 gene. When rice plants are exposed to UV radiation, expression of phytoalexin gets increased. Owing to its role in phytoalexin synthesis, OsMAPKK6 gene was overexpressed in transgenic rice line and the plants could show increased phytoalexin content. Another most important fungal disease of rice affecting the grain yield is sheath blight which is caused by *Rhizoctonia solani*. Depending on the extent of infection, crop stage at which it is infected and favourable environmental conditions, sheath blight results in crop losses of up to 50%. The self-defense system of plants is activated when the plant is encountered by any pathogen which leads to production of several PR proteins like chitinases, glucanases, thaumatin-like proteins, etc. Chitinase gene cloned in rice provides resistance against sheath blight. In an attempt to develop sheath blight resistance in rice, basic chitinase gene and a ribosome-inactivating protein were coexpressed in rice and the resulting transgenic plants showed a significant reduction in the incidence of sheath blight.

Resistance to Viral Diseases

Several viruses such as Rice dwarf virus (RDV), Rice black streaked dwarf virus (RBSDV), Rice stripe virus (RSV), Rice tungro bacilliform virus, Rice tungro spherical virus, etc. affects the rice crop and the result is a significant loss in yield. Leafhoppers and planthoppers are the major insect vectors for transmission of these viruses. It is more difficult to control the viruses that can multiply in insects and gets transmitted transovarially. There are examples of



severe devastation of rice due to infection with Rice grassy stunt virus (RGSV) and Rice ragged stunt virus (RRSV) recently in Vietnam during 2006–2007 where 485,000 hectares of paddy fields were severely affected. This caused the loss of 828,000 tons of rice costing around US\$120 million. Therefore, viruses are a serious threat to rice production. Considering the magnitude of losses caused by viruses in rice, it is enormously important to manage the viral disease for ensuring global food security. Genetic engineering would pave the way to the solution of these problems and is one of the most promising approaches for improved resistance against diseases and harmful insects. Development of resistance to viruses is generally achieved by using the concept of pathogen-derived resistance. This includes expression of part of the viral genome in plants that effectively prevents or reduces the infection of that particular virus. Based on this strategy, transgenic rice plants were developed to confer resistance to Rice stripe virus and Rice yellow mottle virus (RYMV). Another innate resistance mechanism of viral resistance in plants is RNA interference (RNAi) or RNA silencing where RNA of a virus acts as a potential trigger to induce resistance against viruses. Large number of eukaryotic organisms have evolutionarily conserved RNA interference mechanism which acts in a sequence-specific manner for silencing the gene expression and it is generally induced by dsRNA. Induction of RNAi by dsRNA involves dicing of dsRNA by dicer endonuclease into small interfering RNAs (siRNAs) that are 21–24 nucleotides in length. RNA induced silencing complex (RISC) is then formed by incorporating these small siRNAs. RISC guides either degradation of complementary mRNAs or represses their translation in sequence-specific manner. One of the most efficient ways to produce the virus-specific dsRNA in plants for developing resistance against viruses is to form it in the form of hairpin structure and there are specialized vectors developed for this purpose. Comparatively, use of RNAi approach targeting different viral genes is less effective for developing virus resistance. The resistance level achieved by RNAi varies from complete resistance without any symptoms or delayed symptoms or absence of resistance which depends on target of virus genome (Shimizu et al. Therefore, to achieve strong resistance against viruses in RNAi transgenic plants, it is very important to identify the best target for RNAi. For example, genes coding for nucleocapsid and movement proteins of Rice stripe virus (RSV) were found to be highly effective targets for RNAi in developing RSV resistant plants. Transgenic plants with RNAi construct targeting pC5 (nucleocapsid) or pC6 (movement protein) genes of RGSV showed no proliferation of virus particles. A similar strategy of targeting these two genes by RNAi can be applied to impart resistance against other plant viruses of Tenuivirus genus. Spreading of Rice tungro bacilliform virus (RTBV) which causes rice tungro disease is aided by Rice tungro spherical virus (RTSV). However, RTSV which is also known as Rice waika virus do not cause visible symptoms after infection to rice. Therefore, strategy has been devised to develop significant resistance against RTBV and other related viruses by developing transgenic rice plants that can produce small interfering RNAs targeting RTSV sequences.

Resistance to Insects

Insect pests are more hazardous to agriculture as they directly affect both vegetative as well as productive growth of crops worldwide (Karthikeyan et al, leading to the global loss of 15%. Barley trypsin inhibitor gene was cloned in rice to confer resistance against insects by Alfonso-Rubi et al. Transgenic expression leads to the production of several protein inhibitors in field crops that provide resistance against agriculturally important pests. Some of the examples are transgenic rice expressing cowpea-TI inhibits growth of *Chilo suppressalis* and



Potato II (T/C-I) inhibitor transferred in rice provide resistance against *M. sexta*. These rice plants produced toxins to the level of 0.05% of a total soluble protein and showed significant resistance against striped stem borer (*Chilo suppressalis*) and rice leaf folder (*Cnaphalocrocis mainsails*). Similarly, transgenic plants of aromatic rice varieties Basmati 370 and M 7 containing Cry II(a) showed resistance to yellow stem borer (*Scirpophaga incertulas*) and rice leaf folder. The cry family of gene contains several different kinds of cry proteins functional against different families of insects. Many of these cry proteins have been utilized to develop transgenic plants. For example, Indica and Japonica rice genotypes were transformed with truncated form of Cry1A(b) gene by microprojectile bombardment as well as protoplast systems to impart insect resistance. Transgenic rice plants harbouring Cry1A(c) gene showed considerable resistance against yellow stem borer whereas plants carrying Cry1A(b) gene showed resistance for both yellow stem borer and striped stem borer. Synthetic Cry1A(c) gene has been introduced in some elite rice breeding lines such as PusaBasmati 1, IR64 and Karnal local to develop insect resistance in them. The whole plant assays at vegetative stage and growing on cut stems of these Bt transgenic lines with Bt titres of 0.1% of total soluble protein resulted in 100% mortality of yellow stem borer larvae. Considering the potential of such transgenic rice lines expressing the Bt toxin of *Bacillus thuringiensis*, their commercialization is expected in near future. Various cry genes transferred in rice for genetic improvement and resistance to different categories of insect are given. In addition to leaf-eating insects, sucking pests like Leaf folder (*Cnaphalocrocis medinalis*) and planthoppers (*Nilaparvata lugens* and *Sogatella furcifera*) severely affects rice crop. Among these, brown planthopper (BPH) is severe threat to rice production due to its destructive nature and thus it causes annual loss of billions of dollars. Cultivars developed through traditional breeding shows promising results, however, there is frequent and rapid breakdown of BPH resistance. Therefore, it is necessary to identify novel and effective BPH resistance genes conferring durable resistance. To control these sap-sucking insects, plant lectins are promising proteins and are effective against Hemipteran insects. Therefore, strategy of increasing amount of lectins in plant tissues is one of the potential ways to impart insect resistance in plants. Garlic lectin gene (ASAL) has been evaluated against chewing (lepidopteran) and sap-sucking (homopteran) insects and found providing resistance against these insects. *Allium* leaf agglutinin (ASAL) has insecticidal activities against various insects. Transgenic rice plants developed using the ASAL gene exhibited resistance to planthoppers. Another gene *Galanthus nivalis* (snow drop) agglutinin (GNA) introduced in rice confers resistance against brown planthopper also developed transgenic rice plants showing resistance to sap-sucking brown planthopper (*Nilaparvata lugens*). These homozygous T2 transgenic plants showed strong resistance against hoppers as compared to wild type. The insect bioassay showed reduced survival rate (~74–83%), slow development, and less fecundity. The major advantage of the transgenic plants was no growth penalty or no adverse effect on phenotypes but plants were highly resistant to insects. Bph3 is another important gene for imparting BPH resistance in rice. There are three genes in cluster known as Bph3 which encodes plasma membrane-localized lectin receptor kinases (OsLecRK1–OsLecRK3). These lectin receptor kinases are responsible for broad-spectrum and durable resistance. Thus, Bph3 is the potential genetic resource that can be transferred to susceptible rice varieties by molecular breeding or recombinant DNA technologies to enhance their resistance against BPH and also white back planthopper. BPH29, a resistance gene with B3DNA binding domain showed resistance against



Brown planthopper by activating salicylic acid signalling pathway and represses the jasmonic acid/ethylene-dependent pathway, similar to defense response shown by biotrophic pathogens. A brown planthopper resistance gene, BPH9 was isolated and widely used in rice breeding. Expression of this gene in rice plants confers resistance against BPH and reduces the damages. Interestingly, there is lot of allelic variation observed in this gene locus and a particular allele is responsible for resistance against a particular BPH population. This is critical for rice plants to cope with the continuous evolution of insect populations with varied virulence.

Herbicide Resistance in Rice for Controlling Weeds

Herbicide resistance is the first major achievement using genetic engineering approaches in plants against weeds. One of the major strategies for developing herbicide resistance in plants is to over-express the target gene of the herbicide. This strategy has been utilized to develop glyphosate-resistant transgenic rice plants by over-expressing a native 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*) gene. One of the major concerns for developing herbicide resistance or such novel traits in plants is transgene flow from transgenic plants to related weed species or elite cultivars. For example, in one such case, flow of a transgene from transgenic rice plants resistant to glufosinate to improved cultivars and weed relatives has been observed. The highest frequency of gene was noticed in the case of weedy rice which raises the threats of developing superweeds. Therefore, it is highly necessary to destroy and control the weedy rice in the fields where transgenic herbicide-resistant rice plants are grown. This will ensure the prevention of development of superweeds.

Conclusion

Owing to the severity of damage caused by biotic stresses in rice, their management, and development of biotic stress-tolerant genotypes is an essential part of rice breeding program. Along with molecular breeding, transgenic technology has huge potential for development of stress-tolerant varieties. Transgenic technology has added advantages like less time and horizontal gene transfer, however, the transgenic plants are subjected to biosafety analysis prior to their commercialization. Transgenic plants in rice have been developed for resistance against devastating fungal diseases like rice blast, bacterial diseases like bacterial blight, and viral diseases. Insect resistance and herbicide resistance are also major traits that are introduced in rice plants. Rapid greenhouse and field trials and in-depth biosafety analysis will ensure their safe landing in farmers' field as well as consumers' plate in near future.

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