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Genetic improvement of rice for biotic and abiotic stress tolerance

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Abstract: Rice (*Oryza sativa* L.) is among the most important food crops that provide a staple food for nearly half of the world's population. Rice crops are prone to various types of stresses, both biotic and abiotic. Biotic stresses include insect pests, fungus, bacteria, viruses, and herbicide toxicity. Among abiotic stresses, drought, cold, and salinity are also well studied in rice. Various genes have been identified, cloned, and characterized to combat these stresses and protect rice crops. The identified genes are successfully transformed into rice plants to produce transgenic plants. These transgenic rice plants are being evaluated under field conditions in different countries. Genetic engineering has a very positive impact on improvement of rice crops. The development of rice with improved traits of biotic and abiotic stress tolerance is discussed in this review article. The objective of this review is to provide an overview of recent research and development in the field of rice biotechnology.

Key words: Biotic stress, Bt rice, genetically modified rice, drought, fungal resistance, herbicide tolerance

1. Introduction

Rice (*Oryza sativa* L.) is one of the oldest cultivated crops. It has been cultivated in India and China for several thousands of years (Poehlman and Sleper, 1995). The major cultivated species of rice, *Oryza sativa* (2n = 2x = 24), originated in southern and southwestern tropical Asia. The other species of cultivated rice, *Oryza glaberrima* (2n = 2x = 24), is indigenous to the upper valley of the Niger River and it is cultivated only in western tropical Africa. Twenty-three species and 10 recognized genome types (AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, and HHKK; Gramene: http://www.gramene.org/species/oryza/rice_taxonomy.html) of *Oryza* are recognized. Close relatives of *O. sativa* are the wild perennial species *O. rufipogon* and the wild annual species *O. nivara*. Both are diploid weedy species with the AA genome.

Rice is an important food crop and it needs continuous improvement due to the continuous increase in population. The major objectives to improve rice crops using biotechnological techniques include: 1) high yield potential, 2) early maturity, 3) resistance to lodging and shattering, 4) resistance to stress environments, 5) disease resistance, 6) insect resistance, 7) grain quality, and 8) enhancement of nutritional components.

2. Economic importance of rice

Rice (*Oryza sativa* L.) is the most important crop of world. About 90% of the world's rice is grown in China, India, Pakistan, Japan, Korea, Southeast Asia, and other adjacent areas (USDA, 2014). Outside of Asia, Brazil and the United States produce the largest amounts of rice (Poehlman and Sleper, 1995). Rice is the staple food for over one-half of the world's people (FAO, 2008). In Pakistan, rice is a highly valued food crop and it is also a major export item. It accounts for 3.2% of the total value added in agriculture and 0.7% of the GDP. Area sown for rice is estimated at 2.891×10^6 ha and the production was 7.005×10^6 t in 2014–2015 (Ministry of Finance, 2015). Rice is cultivated in diversified climatic conditions of Pakistan. Basmati rice (Indica) is grown in the traditional rice-growing belt of Punjab Province. In Swat, in high altitude alpine valleys, temperate Japonica rice is grown. In the south of Khyber Pakhtunkhwa, Sindh and Baluchistan provinces, IRRI-type long-grain heat-tolerant tropical rice is mainly grown.

3. Transformation of rice crops

The world will need about 25% more rice by the year 2030 to meet the estimated demand of an increasing global population (Wani and Sah, 2014). One way to meet this challenge is to grow rice on more area, which is difficult due to increasing urbanization and escalating population in

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underdeveloped countries. The other option is to improve varieties and increase per hectare yield by breeding efforts through conventional methods as well as modern biotechnology. Biotechnology has a promise to increase yield as well as decrease crop loss due to various biotic and abiotic stresses (Gelvin, 2010; Ozawa and Takaiwa, 2010; Mahmood-ur-Rahman et al., 2014b).

4. Insect-resistant rice

Insect-resistant crops have revolutionized modern agriculture and have become a major tool of integrated pest management programs, leading to reduction in insecticide use while protecting the environment and human health (Brooks and Barfoot, 2013). Like in other crops, insect-resistant rice plants were also developed about two decades ago (Fujimoto et al., 1993). Now genetically modified rice lines expressing a gene from *Bacillus thuringiensis* (Bt rice plants) are under field trials in various countries (Tu JM et al., 2000; High et al., 2004; Wang et al., 2014). There are numerous reports indicating that Bt rice can minimize losses due to lepidopteran pests in Asia (High et al., 2004). Shu et al. (2000) reported that transgenic rice transformed with a synthetic *cry1Ab* gene was found significantly tolerant to eight lepidopteran insects, including striped stem borer (SSB; *Chilo suppressalis*) and yellow stem borer (YSB; *Scirpophaga incertulas*). Furthermore, two lines from Bt rice plants were highly resistant to lepidopteran pests under field conditions (Kumar et al., 2008; Deka and Barthakur, 2010; Wang et al., 2014). The major milestones in development of insect-resistant rice plants are reviewed in Table 1.

Insect-resistant hybrid rice plants were evaluated in a field in China and were highly tolerant to rice leaf folder (RLF; *Cnaphalocrocis medinalis*) and YSB (Tu JM et al., 2000; Deka and Barthakur, 2010; Chen et al., 2011). Insect-resistant Bt rice has also been produced in Pakistan (Mahmood-ur-Rahman et al., 2007) and in the Mediterranean region (Breitler et al., 2004). Results of recent field trials in both locations showed significant resistance against target insects, i.e. YSB and RLF (Breitler et al., 2004; Bashir et al., 2005; Mahmood-ur-Rahman et al., 2007, 2012, 2013, 2014a, 2014b; Tabashnik et al., 2009) (Table 2). The rice plants were artificially infested (Figures 1A–1C) with the target insects and their attack was measured quantitatively. Some transgenic rice lines/varieties resistant to YSB have also been developed in India (Ramesh et al., 2004). Transgenic rice plants have also been developed in China and their efficacy was tested in the laboratory as well as in the field (Wang et al., 2012; Li et al., 2013; Li F et al., 2014).

Pyramiding of multiple genes against the same pest or a range of pests has proved to be very effective to induce sustainable resistance against insect pests. Research has been carried out to pyramid *cry1Ab* or *cry1Ac* with either

Table 1. Genetic improvement of rice for insect resistance.

S. no.	Gene(s)	Targets	References
1	*cry1Ab* or *cry1Ac*	YSB*, SSB**	Shu et al. (2000)
2	*cry1Aa* or *cry1Ab*	SSB	Breitler et al. (2004)
3	*cry1Ab* and *cry1Ac*	YSB	Ramesh et al. (2004)
4	*cry1Ab*	SSB	Cotsaftis et al. (2002)
5	*cry1Ab*	YSB and RLF***	Bashir et al. (2005)
6	*cry*, *Xa21*, and *RC7*	YSB, bacterial blight, sheath blight	Datta et al. (2003)
7	*gna* and *cry1Ac*	Homopteran, coleopteran, and lepidopteran insects	Nagadhara et al. (2003)
8	*Itr1*	Rice weevil	Alfonso-Rubi et al. (2003)
9	*cry1Ac* and *cry2A*	YSB and RLF	Mahmood-ur-Rahman et al. (2007)
10	Bt and *CpT1*	Insect resistance	Rong et al. (2007)
11	Bt, protease inhibitors, enzymes, and plant lectins	Insect resistance	Deka and Barthakur 2010
12	*cry2Aa*	Insect resistance	Wang et al. (2012)
13	*cry1Ab*	Insect resistance	Wang et al. (2014)

*YSB = yellow stem borer, **SSB = stripe stem borer, ***RLF = rice leaf folder.

Table 2. Field evaluation of transgenic rice.

S. no.	Gene(s)	Trait	Location	References
1	*cry1Ab* and *cry1Ac*	Insect resistance	China	Tu JM et al. (2000)
2	*cry1Ab*	Insect resistance	China	Shu et al. (2000)
3	*cry1Ac* and *cry2A*	Insect resistance	Pakistan	Bashir et al. (2005)
4	*cry1Aa* and *cry1B*	Insect resistance	Spain	Breitler et al. (2004)
5	*cry2A*	Insect resistance	China	Chen et al. (2005)
6	*cry1Ac* and *cry2A*	Insect resistance	Pakistan	Mahmood-ur-Rahman et al. (2007, 2012, 2014a)
7	*cry1Ac* and *CpT1*	Insect resistance	China	Han et al. (2006)
8	*cry1Ab*	Insect resistance	China	Wang et al. (2014)
9	*Xa21*	Bacterial blight resistance	China	Tu J et al. (2000)
10	*bar*	Herbicide resistance	USA	Oard et al. (2000)
11	*bar*	Herbicide resistance	Spain	Messeguer et al. (2004)
12	*bar*	Herbicide resistance	USA	Zhang et al. (2004)

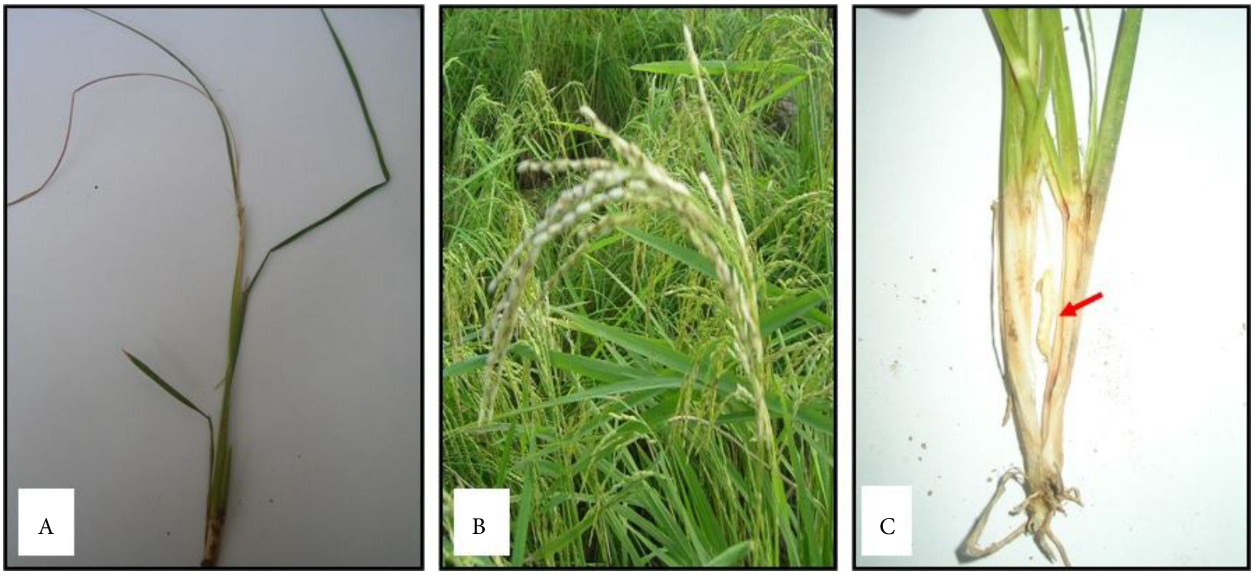

Figure 1. Artificial infestation by yellow stem borer (YSB) in transgenic and nontransgenic rice plants to evaluate the efficiency of Bt genes against target insects. A) Symptoms of YSB attack at the vegetative stage of plant growth; the central tiller is dead due to insect attack, which is called "dead heart". B) Symptoms of YSB attack at the reproductive stage of plant growth; the panicle becomes white and there is no grain development inside, a condition called "white head". C) Artificial infestation by YSB.

cry2A or *cry9C* for improved and sustainable resistance in Bt rice. *cry1Ab* and *cry1Ac* have the same binding site in insects like SSB and YSB (Alcantara et al., 2004) and may be very effective in pyramiding of genes. Use of *cry* genes along with the snowdrop (*Galanthus nivalis*) lectin gene, *gna*, provided elevated levels of tolerance to a broad range of pests, including sucking insects (Ramesh et al., 2004), as the Bt genes have no effect on sucking insect pests (Bernal et al., 2002).

It is important to pyramid *cry* genes with other insecticidal genes to induce sustainable resistance. These non-Bt insect resistant genes may be obtained from plants such as lectins, ribosome-inactivating proteins, or protease inhibitors (Sharma et al., 2003). The lectin gene (*gna*) has been widely used to develop insect-resistant rice for built-in resistance against various species of homopteran, lepidopteran, and coleopteran insect pests (Nagadhara et al., 2003). Transgenic rice strains producing proteinase inhibitor proteins have been developed and evaluated in the field (Mochizuki et al., 1999).

Recent developments such as an inducible and tissue-specific expression of the transgene, pyramiding of multiple genes for a broader range of protection, and delayed resistance development of insect pests could assist in the commercial application of genetically modified rice (Kumar et al., 2008). Protease inhibitors (Hernandez et al., 2003) and lectins are more effective than *gna* (Lopez et al., 2002). The cloning protease inhibitors and lectins have the potential to allow the development of insect-resistant rice (Kumar et al., 2008), which is more effective against sucking insects.

5. Fungus-resistant rice

Genetic improvement of rice for fungus resistance is the need of the day due to vulnerability of this crop to various pathogens, including fungi, bacteria, and viruses (Gust et al., 2010; Miah et al., 2013; Sattari et al., 2014). There are several genes that were isolated and cloned, and then transformation was carried out in rice plants to confer resistance against various species of fungi. The *Pi-ta* gene was recently cloned and reported to have significant resistance against rice blast (Dai et al., 2010; Delteil et al., 2010). Indigenous resistance to sheath blight was also studied and mapped (Liu et al., 2009). The molecular markers were also found by crossing between transgenic and susceptible/nontransgenic varieties (Liu et al., 2009). Indica rice generations harboring the *PR-3* rice chitinase gene were found tolerant to sheath blight (Datta et al., 2003). The *Rir1b* gene is a member of defense-related genes family. It has been identified and characterized only in cereals (Mauch et al., 1998). Transgenic rice containing *Rir1b* was reported to have an enhanced resistance to rice blast (Li et al., 2009).

Several proteins have also been identified as good candidates to confer resistance against various species of fungi in rice plants, which are involved in tolerance of pathogen attack. Examples include lipid transfer protein (Guiderdoni et al., 2002), selenium-binding protein homolog (Sawada et al., 2004), genes taking part in flavonoid pathways (Gandikota et al., 2001), puroindoline proteins (Krishnamurthy et al., 2001), rice homolog of maize HC-toxin reductase (Uchimiya et al., 2002), defensins (Kanzaki et al., 2002), trichosanthins (Yuan et al., 2002), phytoalexins (Lee et al., 2004), protease inhibitor protein genes (Qu et al., 2003), genes involved in cell death (Matsumura et al., 2003), antifungal protein from *Aspergillus flavus* (Coca et al., 2004), and mycotoxin detoxifying compounds (Higa et al., 2003).

6. Bacteria-resistant rice

Bacterial blight-resistant transgenic rice lines were developed by transforming an endogenous gene, *Xa21* (Song et al., 1995). *Xa21* has been introduced into different rice cultivars by genetic transformation as well as through conventional breeding techniques (Tyagi and Mohanty, 2000). Transgenic plants with *Xa21* were evaluated under field conditions with promising results (Tu J et al., 2000). This gene was found to be the best candidate so far to induce resistance against bacterial blight. It was also reported that the gene could provide multiple stress tolerance along with other genes (Datta et al., 2003). Another resistant gene, *Xa26*, was isolated from rice and encodes a similar protein with similar effects in transgenic plants as the plants having the *Xa21* gene (Sun et al., 2004; Zhang, 2009).

Some other genes have also been transformed to develop bacterial-resistant rice (Zhou et al., 2011). The cecropins are a family of genes having antibacterial activity. They express peptides in the hemolymph of *Cecropia* moths. They have been transformed and expressed in plants with satisfactory results (Huang et al., 1997). Transgenic rice plants producing ferredoxin-like protein, AP1, expressed tolerance to *X. oryzae* (Tang et al., 2001). Rice has also been transformed to induce resistance against *Burkholderia plantarii*. Field studies of rice plants expressing *Xa21* showed a significant increase in yield due to less damage by the pathogen (Tu J et al., 2000). *Xa21* is a good candidate gene to be transformed in rice for sustainable resistance against bacterial diseases and it should be released commercially for the general farming community (Kumar et al., 2008).

7. Virus-resistant rice

Crop damage due to viruses has been a serious problem worldwide for several decades. The risk of damage due to rice viruses, especially rice stripe virus (RSV) and rice dwarf virus (RDV), which caused greater yield losses in the 1960s (Toriyama, 2010), is increasing. The use of insecticides to control the vector insects is one possible way to control rice crops but the high costs of insecticide and the risk to the environment are the major limiting factors. Genetic resistance against rice viruses or their insect vectors is also one of the most effective methods of protecting rice plants from virus infection. Rice plants having resistance against RDV (Shimizu et al., 2009; Sasaya et al., 2013) and RSV (Xiong et al., 2009) were developed and evaluated.

Rice tungro disease is very dangerous viral disease of rice. It is caused by infection by two viruses, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV). Green leafhopper (*Nephotettix virescens*) is the vector of RTSV and assists in the transmission of the virus. Rice plants have been transformed by using the coat protein-mediated resistance strategy. Huet et al. (1999) produced transgenic rice plants containing the RTSV replicase gene. The transgenic plants showed moderate resistance to RTSV.

8. Herbicide-tolerant rice

Herbicide tolerance is an important agronomic trait that has been used to control weeds very efficiently for several decades. Many genes are being used to develop herbicide-tolerant plants, including the *bar* gene and the *EPSPS* gene. The *bar* gene is isolated from *Streptomyces hygroscopicus*, which detoxifies herbicide glufosinate, while the *EPSPS* gene is isolated from *Agrobacterium* strain CP4 and detoxifies glyphosate herbicides (Kumar et al., 2008). Herbicide-resistant GM rice plants encoding the *bar* gene were produced during the early stages of rice transformation research (Tyagi and Mohanty, 2000) and are currently under field trials (Oard et al., 2000).

Considerable efforts have been carried out to identify other sources of genes that could be used for sustainable resistance against herbicides in transgenic rice. One such example is *Bacillus subtilis* protoporphyrinogen oxidase, which is transformed in rice plants and is an efficient source of resistance against oxyfluorfen herbicide (Jung et al., 2004). Other example is human cytochrome P450s; overexpression in rice showed variable responses to herbicides (Kawahigashi et al., 2003).

9. Abiotic stress-tolerant rice

Abiotic stresses including drought, high and low temperatures, salinity, submergence, and oxidative stress contribute significantly to reduce crop yield. More than 50% crop damage has been reported due to these stresses worldwide (Bray et al., 2000; Iqbal et al., 2013; Li Y et al., 2014). They are often interlinked and cause similar cellular as well as physiological damage. Moreover, they also activate similar cell-signaling pathways (Nakashima et al., 2009; Qin et al., 2011). Several proteins, antioxidants, and compatible solutes are produced in response to stress conditions. Many crop plants have been developed by overexpression of genes responsible for these compounds and evaluated for various abiotic stresses under laboratory and field conditions (Luo et al., 2010).

Tolerance to water shortage and salt stress are the most damaging factors that inhibit yield in rice crops. GM technology is one of the available options to increase abiotic stress tolerance in crop plants (Flowers, 2004). Development of transgenic plants containing various genes that induce tolerance to drought and/or salinity tolerance in different plants (Tyagi and Mohanty, 2000; Iqbal et al., 2013) has been extended to crop species such as rice (Table 3). Furthermore, many new genes have been identified and isolated that are responsible for providing salt and drought stress tolerance in model plants (reviewed by Flowers, 2004; Zhang et al., 2004).

Hoshida et al. (2000) transformed rice with the chloroplastic glutamine synthase (*GS2*) gene and successfully developed transgenic plants. Transgenic plants with high *GS2* levels demonstrated an enhanced photorespiration capability and they were highly tolerant to salt stress and chilling stress (Hoshida et al., 2000). Xiong and Yang (2003) isolated and characterized a stress-responsive *MAPK* gene, *OsMAPK5*, from rice whose expression could be regulated by ABA and various other biotic and abiotic stresses. Plants overexpressing *OsMAPK5* showed elevated tolerance to drought, salt, and cold stress (Xiong and Yang, 2003; Osakabe et al., 2014; Savchenko et al., 2014).

Table 3. Abiotic stress-tolerant genes in rice.

S. no.	Gene(s)	Function	Source	Characteristics of transgenic plant	References
1	*GS2*	Glutamine synthase	Rice	Tolerant to salt and cold stresses	Hoshida et al. (2000)
2	*OsCDPK7*	Calcium-dependent protein kinase	Rice	Tolerant to salt and drought stresses	Saijo et al. (2000)
3	*OsMAPK5*	MAP kinase	Rice	---	Xiong and Yang (2003)
4	*Adc*, *Samdc*	Polyamine biosynthesis	*Datura*, oat	Tolerant to salt and drought stresses	Capell et al. (2004)
5	*HVA1*	LEA protein	Barley, wheat	Tolerant to salt and dehydration stress	Babu et al. 2004
6	*OtsA*	Trehalose biosynthesis	*E. coli*	Tolerant to salt, drought, and cold stress	Jung et al. (2003)
7	*p5cs*	Proline biosynthesis	Moth bean	Transgene expressed when stress induced	Hur et al. (2004)
8	*pdc1*, *adc*	Pyruvate decarboxylase	Rice	Submergence tolerance	Rahman et al. (2001)
9	*AGPAT*, *SGPAT*	Fatty acid biosynthesis	*Arabidopsis*, spinach	Improved photosynthesis at low temperatures	Ariizumi et al. (2002)
10	*Cat*	Catalase	Wheat	Cold-tolerant	Matsumura et al. (2003)
11	*spl7*	Heat-stress transcription factor	Rice	Tolerance to heat stress	Yamanouchi et al. (2002)

The dehydration-responsive elements (DREs) regulate the expression of various genes under abiotic stress conditions (Nakashima et al., 2014; Wani and Sah, 2014). Dubouzet et al. (2003) isolated 5 rice *DREB* homologs of *Arabidopsis*, *OsDREB1A*, *OsDREB1B*, *OsDREB1C*, *OsDREB1D*, and *OsDREB2A*, and characterized them in *Arabidopsis*. Researchers generated transgenic rice plants overexpressing various *OsDREB* genes driven by the combination of several promoters. They suggested that *OsDREB1A* proved very useful for the development of transgenic dicot and monocot plants with increased tolerance to drought, salt, and/or cold stresses (Wang et al., 2007; Nawaz et al., 2014).

The *ACS* gene is responsible for submergence resistance in rice plants. The mRNA level was found to be higher under completely submerged conditions in vascular bundles of young stems and leaf sheaths. Overexpression of the *YK1* gene in rice also resulted in the same results (Uchimiya et al., 2002). A transcription factor (*Spl7*) was identified and cloned in rice plants. Its overexpression in *spl7* mutants inhibited leaf spot development induced by high temperature (Yamanouchi et al., 2002). Heat-tolerant rice plants have also been developed by overexpressing either heat shock proteins (Murakami et al., 2004) or enzymes involved in oxidative stress tolerance (Kouril et al., 2003).

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