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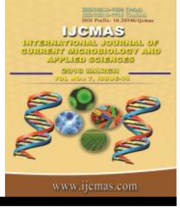

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Recent Advances in Transgenic Rice Research Technology

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ABSTRACT

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Rice is an important staple food crops for more than half of the world's population. Rice (*Oryza sativa*) productivity is adversely impacted by numerous biotic and abiotic factors. An approximate 50% of the global rice production is hampered annually due to the damage caused by biotic factors, out of which ~20% is due to the attack of insect pests. Insect pests are a major biotic constraint on rice production. Transgenic approach has been explored to confer insect pest resistance to rice plants. In this paper, we review the progress that has been made in last years insect resistant transgenic rice research.

Introduction

Rice (*Oryza sativa L.*) is one of the most important sources of calories to human population providing 23% calories globally (Khush, 2003). It is the third largest produced cereal crop after wheat and maize. It is estimated that by 2050 the world would be requiring 60% more food, feed and biomass from the same amount of the land (FAO Report, 2009). Among cereals and monocots, rice being one of the major food crop of the world, having relatively small genome size (389 Mb, International Rice Genome Sequencing Project, 2005), complete genome sequenced, with well-developed markers emerged as a model monocot system for studying gene expression and function and for genetic transformation (Tyagi *et al.*, 1999; Giri and Laxmi, 2000; Bajaj and Mohanty, 2005). Green revolution has increased the global rice production from 257 million tons to 718 million tons since 1996 to 2011 (Wani and Sah, 2014). But still the food grain production is lagging behind due to increased demand due to several factors including continuous increase in global population, urbanization. The condition is further deteriorated by continuously decreasing arable land for crops, abiotic and biotic stress amongst many other factors. The two major biotic stresses, which greatly constrain rice production, are insect pests and weeds. In this regard plant tissue culture and transgenic approaches are powerful means to address these challenges by transferring target genes to host organisms through different strategies. The objective of this review is to trace the advancements in the field of transgenic insect resistant rice.

Transgenic rice for insect resistance

Insects not only damage the rice plant physiologically, but also act as a vector for various viral diseases (Kathuria *et al.,* 2007). The leaf folders, plant hoppers, stem borers and gall midges are most common insect pest species (Maclean *et al.,* 2002). Insect pest attack can cause rice yield loss up to 10% every year (Chen *et al.,* 2009). As chemical insecticides pose risk to both environmental as well as human health, thus genetic engineering provide a safe way to develop insect resistance in rice by transferring genes from different sources. The genes from *Bacillus thuringiensis* have been used extensively in this regard. Majority of the *Bacillus thuringiensis*'s cry proteins are harmful to Lepidopteran insect pests; however, some of them are also lethal to Coleopteran insect pests from (McPherson *et al.,* 1988) or Diptera (Yamamoto and Mclaughlin, 1981). It has been reported that Bt proteins do not pose any risk to beneficial insects, other animals, or humans (Klausner, 1984).

There are different reports, in which *Bacillus thuringiensis* (*Bt*)-derived genes namely *Cry* genes have been introduced in rice against insect resistance via different methods of gene delivery electroporation (Fujimoto., 1993), biolistic method (Wunn *et al.,* 1996; Nayak *et al.,* 1997; Ghareyazie 1997; Alam *et al.,* 1998; Datta *et al.,* 1998; Alam *et al.,* 1999); and by *Agrobacterium* method (Cheng *et al.,* 1998). Fujimoto *et al.,* (1993) were the first to successfully generate the insect-resistant transgenic rice by transferring modified *cry1Ab* gene from *Bacillus thuringiensis*. Subsequently, several rice varieties were successfully transformed using variants of *cry* gene as shown in Table 1. In India, first transgenic rice expressing *cry* gene viz IR62, was produced Nayak *et al.,* (1997). Cheng *et al.,* (1998) produced transgenic rice plants via *Agrobacterium*-mediated transformation using two *Bt* gene synthetic *crylA(b)* and *crylA(c)*. Maqbool *et al.,* (1998) introduced novel endotoxin *cry2A* gene in Basmati 370 and M7 cultivars via particle bombardment method. Shu *et al.,* (2000) successfully obtained highly pest resistance using synthetic *cry1Ab* that showed resistance toeight lepidopteran pests, both in laboratory and natural conditions. However transgenic plants showed some variation in agronomic traits such as seedling growth and yield (Shu *et al.,* 2002). Chen *et al.,* (2005) produced transgenic *Indica* rice with enhanced resistance against lepidopteron pests using synthetic *cry2A* of *Bacillus thuringiensis.* Tang *et al.,* 2006 developed insect-resistant transgenic indica rice variety namely, Minghui 63 with a synthetic *cry1C* gene. The resistant gene, *cry1C*, driven by the rice rbcS promoter (small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase), was introduced into Zhonghua 11 (*Oryza sativa* L. ssp. *Japonica*) via *Agrobacterium*-mediated transformation (Ye *et al.,* 2009). Yarasi *et al.,* (2008), Sengupta *et al.,* (2010), Chandrasekhar *et al.,* (2014) produced transgenic plants resistant to sap-sucking insects viz., brown planthopper (BPH), green leafhopper (GLH) and whitebacked planthopper (WBPH) by transferring *Allium sativum* leaf lectin gene (*asal*) from garlic, coding for mannose binding homodimeric protein (ASAL) through *Agrobacterium*-mediated genetic transformation of embryogenic calli. Weng *et al.,* (2014) used male sterile lines as transformation materials and the modified/optimized *Cry2Aa* gene along with *Bar* gene as a selection maker was transferred into 4008S via *Agrobacterium*-mediated method to generate insect-resistant and herbicide-tolerant Photoperiod-sensitive genic male sterile rice. Yang *et al.,* (2014) generated transgenic *rice “Xiushui 134”* that is highly resistant to leaf folder using *cry1Ac1* by *Agrobacterium* mediated transformation method. Jin *et al.,* (2015) developed a highly lepidopteran pest resistant *japonica* rice variety Jijing 88 by transferring synthetic *cry2A* gene via *Agrobacterium*-mediated transformation. Recently, Manikandan *et al.,* (2016) successfully engineered rice plants expressing synthetic *cry2AX1* gene exhibits resistance to rice leaf folder. Lee *et al.,* 2016 produced insect resistant rice transgenic plants by using the construct with the insecticide *cry1Ac* gene for use in practical agriculture. Similarly, Ling *et al.,* (2016) successfully developed marker-free RSV-resistant transgenic plants using a twin T-DNA system and RNAi technology, a synthetic *cry2A* gene were introduced via *Agrobacterium* mediated cotransformation into an elite *indica* restorer line Minghui 86 (*Oryza sativa* L. ssp. *indica*).

Table.1 Examples of insect-resistant transgenic rice developed over last few years

Variety	Method of Transformation	Gene	References
IR-64	Agrobacterium-mediated	ASAL	Sengupta et al., 2010
Tachisugata	Agrobacterium-mediated	*DB1/ G95A-mALS*	Yoshimara et al., 2011
Nanjing 45	*Agrobacterium*-mediated	*sbk* (modified from *Cry1A(c)*) and *sck* (modified from *CpTI*)	Zhang et al., 2013
IR-64	Agrobacterium-mediated	ASAL	Chandrasekhar et al., 2014
4008S	Agrobacterium-mediated	*Cry2Aa*	Weng et al., 2014
Xiushui 134	Agrobacterium-mediated	*cry1Ac1*	Yang et al., 2014
Jijing 88	Agrobacterium-mediated	*cry2A*	Jin et al., 2015
ASD16	Agrobacterium-mediated	*cry2AX1*	Manikandan et al., 2016
		cry1Ac	Lee et al., 2016
Minghui 86	*Agrobacterium* mediated	*cry2A*	Ling et al., 2016
Fuhui 838	*Agrobacterium* mediated	*(pta) and (Bt)*	Cui et al., 2016

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. In addition a number of genes other than *cry* genes *viz.* plant lectins and proteinase inhibitor genes have been exploited for generation of insect resistant transgenic crops. Cui *et al.,* (2016) successfully obtained broad-spectrum and high insect-resistant transgenic rice by transferring bivalent plant expression vector carrying two insect-resistant genes *viz.,* *Pinellia ternate* agglutinin gene (*pta*) and *Bacillus thuringiensis* gene (*Bt*) into rice restorer Fuhui 838. Similarly, highly enhanced insect resistance has been obtained by Zhang *et al.,* (2013) by transferring bivalent plasmid of pCDMARUBA-*Hyg*, containing two insect-resistance genes, *sbk* (modified from *Cry1A(c)*) and *sck* (modified from *CpTI*) in super japonica rice Nanjing 45 by *Agrobacterium*-mediated transformation method.

The commercialization of insect resistant transgenic rice would help in increasing the rice production globally as well as in meeting the ambition of food security. Commercialization of insect resistant transgenic rice requires proper field trials. In addition, to reduce the development of insect resistance to transgenic crops, there is an urgent need to explore the strategies for delaying resistance such as integrated pest management as well need to explore alternative sources for pest resistance other than cry genes such as plant lectins.

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