Recent Advances in Transgenic Rice Research Technology

Nayan Tara¹, Meena²* and Manjjri Singal¹

¹Department of Biotechnology, CRM Jat College, Hisar-125004, Haryana, India
²Microbial Resource Technology Laboratory, Dept. of Microbiology, Kurukshetra University Kurukshetra-136119, India

*Corresponding author

A B S T R A C T

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.

Keywords

Oryza sativa, Transgenic rice, Biotic stress

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., 1997). 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 cry1A(b) and cry1A(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 to eight lepidopteron 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 |
| Minghui 86 | Agrobacterium mediated | cry1Ac | Lee 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.

References

Alam, M. F., Datta, K., Abrigo, E., Oliva, N., Tu, J, Virmani S S, Datta S.K. 1999. Transgenic insect resistant maintainer line (IR68899B) for improvement of hybrid rice. Plant Cell Rep., 18:572-575.

Alam, M.F., Datta, K., Abrigo, E., Vasquez, A., Senadhira, D., Datta, S.K. 1998. Production of transgenic deepwater indica rice plants expressing a synthetic Bacillus thuringiensis cryIA(b) gene with enhanced resistance to yellow stem borer. Plant Science., 135: 25-30.

Bajaj, S. and Mohanty, A. 2005. Recent advances in rice biotechnology towards genetically superior transgenic rice. Plant Biotechnol. J., 3:275-307

Bharathi, Y., Vijaya, K.S., Pasalu, I.C., Balachandran, S.M., Reddy, V.D. et al., 2011. Pyramided rice lines harbouring Allium sativum (asal) and Galanthus nivalis (gna) lectin genes impart enhanced resistance against major sap-sucking pests. J Biotechnol.,152:63–71

Chandrasekhar, K., Vijayalakshmi, M., Vani, K., Kaul, T. and Reddy, M.K. 2014. Biotechnology Letters., 36(5):1059-67. doi: 10.1007/s10529-014-1459-8.

Chen, H., Lin, Y., and Zhang, Q. 2009. Review and prospect of transgenic rice research. Chin. Sci. Bull., 54, 4049–4068. doi: 10.1007/s11434-009-0645-x

Chen, H., Tang, W., Xu, C.G., Li, X.H., Lin, Y.J., Zhang, Q.F. 2005. Transgenic indica rice plants harboring a synthetic Cry2A* gene of Bacillus thuringiensis exhibit enhanced resistance against lepidopteran rice pests. Theoretical and Applied Genetics., 111: 1330–1337.

Cheng, X., Sardana, R., Kaplan, H., Altosbaar, I. 1998. Agrobacterium transformed rice plants expressing synthetic cryIAb and cryIAc genes are highly toxic to striped stem borer and yellow stem borer. Proc. Natl. Acad. Sci., USA. 95: 2767-2772.

Cui, Y.Q., Li, S.W., Zhang L., Hong, F.; Li Y.F., Zha, R.M. 2016. Breeding and preliminary identification of binary insect-resistant transgenic rice. Journal of Southern Agriculture., 47 (2):169-173.

Datta, K., Vasquez, A., Tu, J., Torrizo, L., Alam, M.F., Olivia, N., Abrigo, E., Khush, G.S., Datta, S.K. 1998. Constitutive and tissue-specific differential expression of cryIAb gene in transgenic rice plants conferring resistance to rice insect pest. Theor. Appl. Genet., 97: 20-30

Dossa, G.S., Oliva, R., Maiss, E., Vera-Cruz, C., Wydra, K. 2016. High temperature enhances the resistance of cultivated African rice, Oryza glaberrima, to bacterial blight. Plant Dis., 100: 380-387.

FAO. 2009. Production Year Book for 2011. UN. Rome, Italy p: 118.

Fujimoto, H., Itoh, K., Yamamoto, M., Kyozuka, J., Shimamoto, K. 1993. Insect resistant rice generated by introduction of a modified δ-endotoxin gene of Bacillus thuringiensis. Bio/Technology., 11: 1151-1155

Ghareyazie, B., Alinia, F., Menguito, C.A., Rubia, L.G., de Palma, J.M., Liwanag, E.A., Cohen, M.B., Khush, G.S., Bennett, J. 1997. Enhanced Resistance to Two Stem Borers in Aromatic Rice Containing a Synthetic cryIAb Gene. Mol. Breed., 3: 401-414.

Giri, C. and Vijaya Laxmi, G. 2000. Production of transgenic rice with agronomically useful genes: an assessment. Biotech Adv., 18: 653-683.
International Rice Genome Sequencing Project. 2005. The map-based sequence of the rice genome. *Nature.*, 436: 793-800.

Jiang, Y., Sun, L., Jiang, M., Li, K., Song, Y. and Zhu, C. 2013. Production of marker-free and RSV-resistant transgenic rice using a twin T-DNA system and RNAi. *J. Biosci.*, 38: 573–581] DOI 10.1007/s12038-013-9349-0

Jin, Y.M., Rui, M.A., Zhi, Jing, Y.U., Ling, W et al., 2015. Development of lepidopteran pest-resistant transgenic japonica rice harboring a synthetic cry2A* gene. *Journal of Integrative Agriculture.*, 14. 10.1007/S12038-011-0311-9

Khush, G.S. 2003. Productivity improvement in rice. *Nutritional Review.*, 61: 114-116.

Klausner, A. 1984. Microbial insect control: using bugs to kill bugs. *Nat Biotechnol.*, 2: 408–419

Lee, D.K., Park, S.H., Seong, S.Y. et al., 2016. *Plant Biotechnol Rep.*, 10:391. https://doi.org/10.1007/s11816-016-0410-y

Ling, F., Zhou, F., Chen, H., Lin, Y. 2016. Development of Marker-Free Insect-Resistant Indica Rice by *Agrobacterium tumefaciens*-Mediated Co-transformation. *Frontiers in Plant Science.*, 7:1608. DOI=10.3389/fpls.2016.01608

MacLean, J.L., Dawe, D.C., Hardy, B., Hettel, G.P. 2002. Rice Almanac. International Rice Research Institute, Metro Manila., 13–14.

Manikandan, R., Balakrishnan, N., Sudhakar, D., Udayasuriya, V. 2016. Transgenic rice plants expressing synthetic cry2AX1 gene exhibits resistance to rice leaffolder (*Cnaphalocrosis medinalis*). *Biotech.*, 6(1): 10. doi: 10.1007/s13205-015-0315-4

Maqbool, S.B., Husnain, T., Raizuddin, S., Christou, P. 1998. Effective control of yellow rice stem borer and rice leaf folder in transgenic rice indica varieties Basmati 370 and M 7 using novel delta-endotoxin cry2A *Bacillus thuringiensis* gene. *Mol Breed.*, 4: 501-507.

McPherson, S.A., Perlak, F.J., Fuchs, R.L., Marrone, P.G., Lavrik, P.B., Fischhoff, D.A. 1988. Characterization of the coleopteran specific protein gene of *Bacillus thuringiensis* var. tenebrionis. *Biotechnology.*, 6: 61–66.

Mukhtar and Hasnain. 2017. Transgenic basmati rice transformed with the Xa21 gene shows resistance against bacterial leaf blight. *Turkish Journal of Botany.*, 41: 1-10

Nayak, P., Basu, D., Das, S., Basu, A., Ghosh, D., Rasmakrishnan, N.A., Ghosh, M., Sen, S.K. 1997. Transgenic elite indica rice plants expressing crylAc borer (*Scirpophaga incertulas*). *Proc. Natl. Acad. Sci. USA.* 94: 2111-2116.

Sengupta, S., Chakraborti, D., Mondal, H.A., Das, S. 2010. *Plant Cell Rep.*, 29(3):261-71. doi: 10.1007/s00299-010-0819-7.

Shu, Q., Ye, G., Cui, H. et al., 2000. Transgenic rice plants with a synthetic crylAb gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species *Molecular Breeding.*, 6: 433. https://doi.org/10.1023/A:1009658024114

Shu, Q.Y., Cui, H.R., Ye, G.Y., Wu, D.X., Xia, Y.W., Gao, M.W., Altosaar, I. 2002. Agronomic and morphological characterization of *Agrobacterium* transformed Bt rice plants. *Euphytica.*, 127: 345-352.

Song, W.Y., Wang, G.L., Chen, L.L., Kim, H.S., Pi, L.Y., Holsten, T., Wang, B., Zhai, W.X., Zhu, H., Fauquet, C. et al., 1995. A receptor kinaselike protein encoded by the rice disease resistance gene, Xa21. *Science.*, 270: 1804-1806.

Tang, W., Chen, H., Xu, C.G., Li, X.H., Lin, Y.J., Zhang, Q.F. 2006. Development of insect-resistant transgenic *indica* rice with a synthetic *Cry1C* gene. *Molecular Breeding.*, 18: 1–10.
Tu, J., Ona, I., Zhang, Q., Mew, T.W., Khush, G.S., Datta, S.K. 1998. Transgenic rice variety ‘IR72’ with Xa21 is resistant to bacterial blight. Theor Appl Genet., 97: 31-36.

Tyagi, A.K., Mohanty, A., Bajaj, S., Chaudhury, A., Maheshwari, S.C. 1999. Transgenic rice: A valuable monocot system for crop improvement and gene research. Crit. Rev. Biotechnol., 19: 41-79.

Wani, S.H. and Sah, S.K.. 2014. Biotechnology and Abiotic Stress Tolerance in Rice. J Rice Res., 2: e105.

Weng, L., Deng, L., Lia, F., Xiao, G. 2014. Optimization of the Cry2Aa gene and development of insect-resistant and herbicide-tolerant photoperiod-sensitive genic male sterile rice. Czech J. Genet. Plant Breed., 50: 19–25.

Wunn, J., Kloti, A., Burkhardt, P.K., Biswas, G.C., Launis, K., Iglesias, V.A., Potrykus, I. 1996. Transgenic Indica rice breeding line IR58 expressing a synthetic cryIAb gene from Bacillus thuringiensis provides effective insect pest control. Bio/Technology., 14: 171-176.

Yamamoto, T. and McLaughlin, R.E. 1981. Isolation of a protein from the parasporal crystal of Bacillus thuringiensis var. kurstaki toxic to the mosquito larva Aedes taeniorhynchus. Biochem Biophys Res Commun., 103: 414–421.

Yang, R.F., Bai, J.J., Piao, Z.Z., and Lee, G.S. 2014. Development of Insect-resistant Transgenic Rice with cry1Acl Gene. Bt Research., 5(4): 1-9 (doi: 10.5376/bt.2014.05.0002)

Yarasi, B., Sadumpati, V., Immanni, C.P., Reddy, V.D., Rao, K.V. 2008. Transgenic rice expressing Allium sativum leaf agglutinin (ASAL) exhibits high-level resistance against major sap-sucking pests. BMC Plant Biol., 8:102.

Ye, R., Huang, H., Yang, Z., Chen, T., Liu, L., Li, X., Chen, H., Lin, Y. 2009. Development of insect-resistant transgenic rice with Cry1C free endosperm. Pest Manag Sci., 65:1015-1020.

Yoshimura, S., Komatsu, M., Kaku, K., Hori, M., Ogawa, T., Muramoto, K., Kazama, T., Ito, Y., Toriyama, K. 2012. Production of transgenic rice plants expressing Dioscorea batatas tuber lectin 1 to confer resistance against brown planthopper. Plant Biotechnol, 29: 501–504

Zhang, Q.J. Li, C., Liu, S.K., Lai, D., Qi, Q.M., Lu, C.G. 2013. Breeding and Identification of Insect-Resistant Rice by Transferring Two Insecticidal Genes-sbk and sck. Rice Science, 20 (1): 19-24

How to cite this article:

Nayan Tara, Meena and Manjjri Singal. 2018. Recent Advances in Transgenic Rice Research Technology. Int.J.Curr.Microbiol.App.Sci. 7(03): 3552-3557. doi: https://doi.org/10.20546/ijemas.2018.703.408