

Review Article

‘Master switch’ genes for disease resistance in rice: lessons learnt and lessons to learn

Pooja Singh^a, Raksha Bawankar^a, K.M. Gothandam^a, Rathinasamy Subashkumar^b, Govindasamy Vivekanandhan^b, Tha. Thayumanvan^c and Subramanian Babu^a

^aSchool of Biosciences and Technology, VIT University, Vellore 632014, India

^bPG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore 641029, India

^cSchool of Biotechnology, Dr.GRD College of Science, Coimbatore 641014, India

Corresponding author e-mail: babu.s@vit.ac.in

Evolving disease resistance rice variety has become a continuous encounter from plant pathologists’ point of view, against the changing scenario in the severity of various fungal, bacterial and viral diseases. Although we are successful to some extent in developing some varieties through conventional and molecular breeding, the recent genome level data indicate that we have only disclosed the tip of an iceberg. The strategy of introducing single foreign gene for every disease crop plants like rice, suffers not only from regulatory issues of genetically modified rice but also lack of sustainability under field tests. This is attributed to the fact that pathogen resistance is mediated by complex networking of genes and their protein products. Transcription factors that control expression of hundreds of other genes offer themselves as valid candidate for key gene transgenics. Rice transcription factors like *Osmyb4* is observed to regulate many PR protein genes and genes involved in post infection defense. Hence, over-expressing these gene(s) in cultivated variety(s) would result in transgenic rice resistant to multiple diseases. The level of constitutive expression of these genes should however be evaluated in major cultivated varieties of rice and a correlation of their native level of resistance/susceptibility to diseases may complement the approach.

Keywords: Transcription factors, rice, regulatory genes, plant defense

Single gene transfer conferring resistance is slowly becoming an out of time strategy as the ‘omics’ era has started to disclose the network, co-ordination and regulation of genes and proteins for every trait including disease resistance. Developing transgenic rice using one or more genes to confer resistance to each disease in isolation is valuable but it can be less sustainable and also misleading because the defense response

form part of complex networks, as disclosed by the accumulating genome, transcriptome and proteome data out of plant-pathogen interaction studies in rice.

The recent transcriptome, proteome and genome analysis in rice related to disease resistance have resulted in identification of variety of genes in the disease responsive pathway and their regulators. The task of genetically engineering rice with all the genes

in pathway is quite impossible and hence would be appropriate to target over-expression of key regulatory genes like transcription factors and others that controls many defense genes. The key questions are 1) What is the constitutive level of expression of these regulatory genes in the cultivated varieties? Can the differential level be correlated with their differential phenotypic reaction to diseases? 2) Would the engineered regulatory genes (engineered to over-express) offer multiple pathogen resistance, if cleverly cloned and evaluated? In the light of these questions, we discuss here some of the so far reported promising regulatory genes for disease resistance in rice.

Rice transcription factors in disease resistance

Transcription factors (TFs) regulate genome expression in response to environmental and physiological signals. TF is encoded by a single gene but regulates the expression of several other genes leading to the activation of complex adaptive mechanisms and hence represents major molecular targets to genetically improve the tolerance of crop plants against different stresses (Khong *et al.*, 2008). This review has illustrated how the elucidation of the function of these TFs can be used to set up genetic engineering strategies and to rationalize molecular breeding using molecular assisted selection towards enhancement of rice tolerance to various stresses. Rather than genetically engineering the rice with single resistant or defense gene for each disease, if a regulatory gene like *Osmvb4* transcription factor is made to express constitutively, there is every possibility that the PR proteins, phenyl propanoid pathway proteins will be controlled and the plant will be resistant to multiple disease. Rice *Osmvb4* is also reported to confer systemic acquired resistance (SAR) against fungi, bacteria and viruses by inducing expression of PR proteins and their activators (Vannini *et al.*, 2006).

Hence *Osmvb4* appears as a wonder gene to engineer and express in high levels to achieve our dream of developing multiple disease resistant rice.

The rice *Osmvb4* gene coding for a MYB transcription factor, its overexpression in *Arabidopsis thaliana* plants induces a high level of tolerance to cold and freezing and results in multiple biochemical changes, commonly observed in plants during cold acclimation (Vannini *et al.*, 2004; Mattana *et al.*, 2005). The microarray analysis of transgenic plants versus the WT showed that the Myb4 activates genes involved in tolerance to different abiotic stresses (such as drought and salt), as well as in pathogen resistance, indicating that this transcription factor is able to integrate the activation of multiple components of the stress response (Vannini *et al.*, 2006). Vannini *et al.* (2007) developed transgenic tomato plants over-expressing rice *Osmvb4* and observed the plants acquiring higher tolerance to drought stress and viral disease. Myb4 protein is involved in different signal transduction pathways and regulated responses not only to abiotic but also to biotic stresses. In order to verify this hypothesis also in tomato, the same research group tested the transgenic line resistance to pathogens. A significant reduction in the symptom severity was observed in tomato plants inoculated with the ToMV. Park *et al.* (2010) dissected the *Osmvb4* network and determined that the *Osmvb4* controls a hierarchical network comprised of several regulatory sub-clusters associated with cellular defense and rescue, metabolism and development. The network activity enhanced cellular antioxidant capacity through radical scavenging mechanisms and increased activities of phenylpropanoid and isoprenoid metabolic processes involving various abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), ethylene and reactive oxygen species (ROS) responsive genes.

As discussed briefly about the regulation of transcription factors with respect to stress modulation, there are other regulatory genes which confer utmost importance in the development of pathogen resistance and also have different proteins involved in overcoming abiotic stress. Two host transcription factors, RF2a and RF2b have been found to play a key role against Rice tungro disease (RTD). They regulate the expression of RTBV promoter and have importance in plant development (Dai *et al.*, 2008).

Enzyme coding regulatory genes

Plant aspartic proteases (AP) are found to be associated with the recognition of pathogen and pests and stimulation of effective defense responses. The gene encoding AP is OsCDR1 isolated from rice expressed ectopically in *Arabidopsis* conferred resistance against bacterial and fungal pathogen (Prasad *et al.*, 2009). In a similar way, the dual function of a rice disease resistance responsive gene, OsDR8 was studied for disease resistance as well as thiamine accumulation. Silencing of the OsDR8 *via* RNA interference resulted in the changes at the phenotypic level. The plant showed susceptibility to *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe grisea*. Also the resulted product of the gene OsDR8 was reported to be homologous to an enzyme which is found to be involved in the biosynthesis of the thiazole, precursor of thiamine; which may be a key factor for the bacterial blight and blast resistance (Wang *et al.*, 2006).

Serine carboxypeptidase proteins (SCPLs) group of protein hydrolyzing enzyme have been reported to have role in multiple cellular processors. OsBISCPL1 gene contains a conserved peptidase S10 domain, serine active sites and signal peptide at N-terminus. This gene is expressed ubiquitously in rice. Expression in leaves has been found to

be up-regulated with the treatment of benzothiadiazole, salicylic acid, jasmonic acid and 1-aminocyclopropanone-1-carboxylic acid and also with the interaction between the rice plant and *Magnaporthe grisea*. Transgenic line of *Arabidopsis thaliana* developed with OsBISCPL1 showed the expression of defense related protein also contributing in the increased level of oxidative stress (Liu *et al.*, 2008).

WRKY protein genes

WRKY proteins are found to exhibit defense against pathogens. OsWRKY6 encodes DNA binding protein that contains one WRKY domain, which is a C₂H₂ type zinc finger motif. This gene acts as transcriptional activator in yeast. It has found to exhibit transcriptional regulator in plants (Hwang *et al.*, 2011). WRKY genes have also been described in rice genome whose most of the functions are unexplored. OsWRKY gene has shown a promising effect against disease resistance at the seedling as well as adult stage with no effects at the fertility stages. They are found to activate genes involved in different pathways. OsWRKY13 has been reported to bind at its own promoter region signaling at least three other signaling genes in the SA- and JA- dependent pathways (Qiu *et al.*, 2007). The OsWRKY13- associated disease resistance pathway synergistically interacts *via* OsWRKY13 with glutathione/ glutaredoxin system and also in flavonoid biosynthesis pathway and enhances the biosynthesis of phytoalexins respectively, giving a salient proof of involvement in the mediation of abiotic and biotic stress (Qiu *et al.*, 2008).

WRKY proteins may play a key role in monitoring of the OsWRKY13 up regulated genes and also genes involved in the biotic stress mediated responses adding to the knowledge that MYB and AP2/EREBP protein may also contribute in the control of

OsWRKY13 down regulated genes (Qiu *et al.*, 2009).

RING finger and Zinc finger protein genes

Analysis of rice RING-H2 finger protein gene OsBIRF1 was studied and reported to have pleiotropic effects on the regulation of growth and defense response against various stresses (Liu *et al.*, 2008). These RING finger proteins contribute a very important role in several processes of growth and development and also contributing in abiotic and biotic stresses. OsBIRF1, a rice gene, was isolated and characterized, and was reported to encode 396 amino acid proteins which belongs to the ATL family and have specific RING-H2 finger domain. The expression of the gene was enhanced after treatment with benzothiadiazole, salicylic acid, 1-aminocyclopropane-1-carboxylic acid and jasmonic acid. Transgenic line of tobacco over expressing OsBIRF1 gene exhibited enhanced resistance against abiotic and biotic stress increasing the expression of pathogenicity related proteins (Liu *et al.*, 2008).

The plant defense response is also provoked due to various abiotic stresses and hence the genes and the respective proteins are found to be involved. A zinc finger protein ZFP245 has been found to be expressed in response to drought and cold stress (Huang *et al.*, 2009). This protein is present in the nucleus and confers trans-activation activity. When over expressed in rice, has reported to result in transgenic, tolerant to cold and drought stress; but it showed growth sensitivity against exogenous abscisic acid, increased free proline levels and elevated expression of rice pyrroline-5-carboxylatesynthetase and proline transporter gene under stress condition. Overall, the overproduction of ZFP245 enhanced the antioxidant activity in rice genome. Involvement of zinc finger protein in biotic stress may help understand the possible

cross-talk in the stress response to pathogens. Zinc finger proteins are valid candidate genes for further exploration for disease resistance.

DEAD-box and F-box protein genes

Apart from the transcription factor, few more protein families have been studied, which play some regulatory role in pathogen infections as well as different stresses. DEAD-box protein has been reported, which are found to have role in nearly all types of processes in RNA metabolism. A rice gene, OsBIRH1, has been reported to encode for DEAD-box RNA helicase protein, was explored to study the role against pathogen infection as well as oxidative stress. This gene contain DEAD domain and all conserved motif, one of the very specific quality of the DEAD-box RNA helicases. Treatment with different defense related signals such as benzothiadiazole, salicylic acid, jasmonic acid and 1-aminocyclopropane-1-carboxylic acid up-regulated and activates the expression of OsBIRH1 in rice, during the interaction of the pathogen *Magnaporthe grisea* and the host plant. The study was performed using *Arabidopsis thaliana*, where the gene showed resistance against *Alternaria brassicicola* and *Pseudomonas syringae* pv. tomato DC3000 (Li *et al.*, 2008).

F-box protein has been also found to be responsible for the growth and development also contributing to the environmental stress. Rice has been reported to contain, OsDRF1, F-box protein reported to encode 328 amino acids containing conserved F-box domain. Expression of the gene induced by benzothiadiazole (BTH), also enhanced by infection with *Magnaporthe grisea*, up-regulated the gene in rice plant. Ectopic over expression in tobacco resulted in tolerance against tomato mosaic virus (ToMV) and *Pseudomonas syringae* pv. tabaci, also contributing in the increased level of ABA sensitivity in plants (Cao *et al.*, 2008).

Other regulatory genes

Germin like protein (GLP) have several roles in plant development and defense. OsGLP1 is the germin like protein present in rice. The single copy of the gene is reported to be expressed predominantly in green vegetative tissues. Superoxide dismutase (SOD) domain in OsGLP1 protein documents for SOD activity and explain its effective role in regulating plant height and resistance to disease (Banerjee and Maiti, 2010).

Similarly OsB1HD1 gene was found to be up-regulated, which encodes for BELL Homeodomain transcription factor. The over expression of the gene by benzothiadiazole (BTH) was enough to induce disease resistance. Expression was enhanced after 6hr of inoculation with *Magnaporthe grisea*. The reports suggested particular role of OsB1HD1 in resistance response in rice (Luo et al., 2005).

Concluding Remarks

With regulatory limitations in India in the genetic engineering to bring foreign genes into crop plants, the alternate safer and very appealing approach is the modulation of already existing useful native genes in the crop plants. Since ectopic expression of rice transcription factors has been observed to confer multiple stress tolerance in *Arabidopsis* and tomato, it is more likely that these kind of genes work in their native genomic background (rice). The transgenic rice obtained by such approaches is expected to be resistant to many diseases including fungal, bacterial and viral diseases and thus solve the issues of genetic engineering of rice by pyramiding with many genes for all these pathogens.

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