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Review

Transgenic strategies for improving rice disease resistance

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Rice diseases are among the most significant limiting factors threatening food productivity. Genetic engineering provides promising strategies to develop and improve disease resistance in rice. This mini-review summarizes the recent progress on studies of genetic engineering for improvement of rice disease resistance against fungal, bacterial and viral pathogens. Improvement of virus resistance can be achieved by generating transgenic rice lines with expression of genes encoding viral coat protein or replication enzymes, expression of RNA interference constructs and suppression of insect vectors. Varieties with improved resistance against fungal and bacterial pathogens can be developed through transgenic expression of cloned disease resistance genes, antimicrobial protein genes, and defense signaling genes or through pyramiding different defense genes. Future direction and strategies on genetic engineering of rice disease resistance are also discussed.

Key words: Disease resistance, transgenic rice, genetic engineering.

INTRODUCTION

Rice is one of the leading primary staple foods for the increasing world population, especially in Asia. Diseases are among the key limiting factors that affect rice production, causing annual yield loss conservatively estimated at 5%. More than 70 diseases caused by fungi, bacteria, viruses or nematodes have been recorded on rice. Resistant cultivars and application of chemical pesticides have been widely used for disease control in practice. However, the useful life-span of many resistant cultivars is only a few years, due to the breakdown of the resistance in the face of high pathogenic variability of the pathogen population. Use of pesticides is costly as well as environmentally undesirable. Thus, there is a great need to develop novel strategies providing durable resistance, giving protection for a long time and over a broad geographic area. Such strategies will be particularly important in cases where the source of resistance is not available. The most significant advancement in the area of varietal development for disease resistance is the use of the techniques of genetic engineering to develop transgenic rice resistant to diseases. In this mini-review, we summarize the recent progress on the studies of genetic engineering of rice for improvement of resistance against fungal, bacterial and viral diseases.

ENGINEERING RESISTANCE AGAINST VIRAL DISEASES

At least 20 viruses can infect rice and about 16 of them may seriously affect rice yield. The distribution of each virus is generally restricted to only one of the continents in which rice is grown, e.g. Rice tungro viruses and Rice stripe virus (RSV) in Asia, Rice hoja blanca virus (RHBV) in South-America, and Rice yellow mottle virus (RYMV) in Africa (Fargette et al., 2006). Two strategies, namely protein-mediated and RNA-mediated resistance, have been the underlying principles to develop successful transgenic viral resistance in rice (Sanford and Johnston, 1985). Both strategies depend on the concept of "pathogen-derived resistance", wherein virus-encoded proteins or RNA are used to interfere with crucial steps in the infection cycle.

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Coat protein-mediated resistance

Coat proteins (CP) of viruses are involved in many aspects of virus-related biology, including encapsidation, virus replication, dissemination, and cell-to-cell and/or systemic movement (Callaway et al., 2001). The RSV CP gene was introduced into two japonica varieties and the resultant transgenic plants expressed the CP at high levels and exhibited a significant level of resistance to virus infection (Hayakawa et al., 1992). The Rice tungro spherical virus (RTSV) CP genes *CP1, CP2* and *CP3* were introduced individually or together into rice and transgenic plants accumulated transcripts of the chimeric *CP* genes. Moderate levels of protection to RTSV infection, ranging from 17 to 73% of reduction from virus infection, and a significant delay of virus replication under greenhouse conditions in transgenic plants expressing the *RTSV-CP1, -CP2* and *-CP3* genes singly or together were observed (Sivamani et al., 1999). Transgenic plants expressing genes that encode wild-type *CP*, deleted *CP*, mRNA of the *CP*, or antisense *CP* sequences showed two types of reactions when challenged with RYMV. Most of the transgenic rice plants expressing antisense sequences of the *CP* and untranslatable *CP* mRNA of RYMV exhibited a delay in virus accumulation of up to a week. However, transgenic plants expressing wild-type *CP* gene accumulated high level of virus particles. These suggest that antisense CP and untranslatable CP mRNA induced moderate resistance, whereas transgenic CP enhanced virus infection (Kouassi et al., 2006). The RHBV nucleocapsid protein N gene was introduced into rice and the resultant transgenic plants had a significant reduction in disease development and a significant increase in performance for important agronomic traits when challenged with RHBV. The *N* gene and RHBV resistance in the transgenic plants were inherited in a stable manner. These transgenic lines could become new genetic resources in developing RHBV-resistant cultivars (Lentini et al., 2003).

Replication enzyme-mediated resistance

A transgenic approach using widely grown RYMV-susceptible rice cultivars and a transgene encoding the RNA-dependent RNA polymerase of RYMV was applied (Pinto et al., 1999). Transgenic plants showed resistance to RYMV strains from different African locations and the resistance was stable over at least three generations. The resistance was shown to be derived from an RNA-based mechanism associated with posttranscriptional gene silencing (Pinto et al., 1999). Transgenic rice plants were produced containing the RTSV replicase (*Rep*) gene in the sense or antisense orientation. Plants producing antisense sequences exhibited significant but moderate level of resistance to RTSV. Plants expressing the *Rep* gene in the sense orientation showed 100% resistance to RTSV and accumulated low levels of viral RNA. This RTSV *Rep*-mediated resistance was effective against geographically distinct RTSV isolates (Huet et al., 1999).

RNA interference-mediated resistance

Homology-dependent selective degradation of RNA, RNA interference (RNAi), is involved in several biological phenomena, including adaptive defense against viruses in plants. RNAi-mediated resistance has been demonstrated to be effective to viral diseases in plants. Transgenic rice plants expressing DNA encoding ORF IV of Rice tungro bacilliform virus (RTBV), both in sense and in anti-sense orientation, resulting in the formation of dsRNA, were generated. Specific degradation of the transgene transcripts and the accumulation of small RNA were observed in transgenic plants. It was also found that different resistance responses against RTBV occurred in the transgenic plants expressing dsRNA. In RTBV-O-Ds1 line, there was an initial rapid buildup of RTBV levels, followed by a sharp reduction, resulting in approximately 50-fold lower viral titers. In RTBV-ODs2 line, RTBV DNA levels gradually rose from an initial low to almost 60% of that of the control at 40 days after inoculation (Tyagi et al., 2008).

The non-structural protein Pns12 of Rice dwarf virus (RDV) is one of the early proteins expressed in cultured insect cells, and it is one of 12 proteins that initiate the formation of the viroplasm, the putative site of viral replication. Pns12- and Pns4-specific RNAi constructs were introduced into rice plants and the resultant transgenic plants accumulated specific short interfering RNAs. Progenies of the transgenic rice plants with Pns12-specific RNAi constructs were strongly resistant to RDV infection (Shimizu et al., 2008). These studies demonstrate that RNAi-mediated resistance is a practical and effective way to control viral infection in crop plants. Moreover, ribozyme, a class of small catalytic RNA molecules that possess sequence-specific RNA cleavage activity, was also demonstrated to be useful in engineering viral resistant transgenic plants. Transgenic rice plants expressing a ribozyme gene with 350 nt hybridizing arms directed against RDV S5 mRNA displayed high resistance or delayed and attenuated disease symptoms (Han et al., 2000).

Insect vector suppression-mediated resistance

Most of the rice viruses are transmitted by phloem feeding insect pests, e.g. brown planthopper and green leafhopper of the order hemiptera (Nagadhara et al., 2004). Thus, suppression of insect feeding by transgenic plants can theoretically control transmission and spread of viruses in the field. ASAL (*Allium sativum* agglutinin from leaf), a novel lectin from garlic was previously demonstrated to be toxic towards hemipteran pests

(Bandyopadhyay et al., 2001). Transgenic rice plants overexpressing *ASAL* under the control of phloem-specific promoters at the insect feeding site were constructed. The transgenic plants contained high level of ASAL (1.01% of total soluble protein) and showed adverse effect on survival, growth and populations of brown planthopper and green leafhopper pests. These transgenic ASAL plants also showed reduced incidence of tungro disease, caused by co-infection of green leafhopper-vectored RTBV and RTSV (Saha et al., 2006). This study provides a novel strategy for engineering resistant rice towards insect pests and viral diseases.

ENGINEERING RESISTANCE AGAINST BACTERIAL AND FUNGAL DISEASES

Among all the rice diseases recorded so far, the blast (*Magnaporthe grisea*), bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) and sheath blight (*Rhizoctonia solani*) are the most serious constraints on high productivity. Several strategies have been established for developing and improving rice resistance against fungal and bacterial diseases through transgenic approaches.

Disease resistance genes

The inheritance of resistance against blast and bacterial leaf blight diseases has been extensively studied (Song and Goodman, 2001). Major genes or loci conditioning resistance against these two diseases have been identified and some of these resistance genes or loci have been widely used in breeding programs (Song and Goodman, 2001). Genetic variability for high levels of resistance against sheath blight is lacking and therefore breeding for sheath blight resistance is lagging. During the last decade, more than 10 disease resistance (*R*) genes, e.g. *Pib*, *Pi-ta*, *Pi2*, *Pi9*, *Pi-d2*, *Pi36*, *Pi37* and *Piz-t* for blast resistance and *Xa1*, *Xa3*/*Xa26*, *xa5*, *Xa21* and *Xa27* for leaf blight resistance, have been characterized at molecular and genetic level (Song et al., 1995; Yoshimura et al., 1998; Bryan et al., 2000; Wang et al., 1999; Zhou et al., 2006; Liu et al., 2007; Lin et al., 2007; Chen et al., 2006; Sun et al., 2004; Xiang et al., 2006; Gu et al., 2005; Iyer and McCouch, 2004; Qu et al., 2006). These cloned *R* genes are very useful novel resources for improving blast and leaf blight resistance by means of genetic engineering. Most of the cloned *R* genes confer high level of race-specific resistance in a gene-for-gene manner and thus the resistance is effective against one or a few related races or strains of the pathogens. However, transgenic rice plants overexpressing *Xa21* showed resistance to 29 isolates of *X. oryzae* pv. *oryzae* from eight countries (Wang et al., 1996). *Pi9*, *Pi2* and *Piz* confer broad-spectrum resistance against *M. grisea* and *Pi9* was shown to be highly resistant to 43 *M. grisea* isolates collected from 13 countries (Zhou et al., 2006; Qu et al., 2006). These *R* genes conferring broad-spectrum resistance have great potential in accelerating improvement of widely-used elite rice varieties for high level of disease resistance; for an example, *Xa21* has been introduced into an elite indica variety, IR72, and the engineered IR72 plants showed good performance in disease resistance against *X. oryzae* pv. *oryzae* (Tu et al., 1999).

ANTIMICROBIAL PROTEINS

Pathogenesis-related proteins

Pathogenesis-related (PR) proteins are a class of novel proteins that are synthesized *de novo* and accumulated in plant tissues after pathogen infection. Some of the PR proteins have activities of hydrolytic enzymes including chitinase and β-1,3-glucanase, which can hydrolyze major components of fungal cell walls, chitin and □-1,3-glucan, respectively. Hydrolysis of these fungal cell wall constituents leads to the inhibition of the growth of several fungi *in vitro* (Punja, 2006). Genes encoding chitinase or β-1,3-glucanase from both plants including rice and microbes have been extensively used in generation of transgenic rice resistant to fungal pathogens (Punja, 2006). Transgenic plants constitutively expressing the *Gns1* gene, encoding a 1,3;1,4-β glucanase, accumulated Gns1 protein up to 0.1% of total soluble protein in leaves. The *Gns1*-overexpressing transgenic plants developed many resistant-type lesions on the inoculated leaf, accompanying earlier activation of defense genes *PR-1* and *PBZ1,* when inoculated with virulent *M. grisea* (Nishizawa et al., 2003).

Transgenic plants which constitutively expressed a rice class-I chitinase gene, *Cht-2* or *Cht-3*, showed significant resistance against two races of *M. grisea* (Nishizawa et al., 1999). Transgenic plants over expressing either a rice chitinase or a rice thaumatin-like protein showed enhanced resistance against *R. solani* (Lin et al., 1995; Datta et al., 1999, 2000, 2001). The activity of chitinase in the transgenic plants overexpressing a rice chitinase gene was found to be correlated to the levels of enhanced resistance against *R. solani* (Datta et al., 2001).

Interestingly, hydrolytic enzymes of microbial origin have also been demonstrated to be effective in engineering rice disease resistance against fungal pathogens. A bacterial family 19 chitinase ChiC from *Streptomyces griseus* showed clear inhibition on fungal hyphal extension *in vitro*. Ninety percent of transgenic rice plants expressing *ChiC* had higher resistance against *M. grisea* than non-transgenic plants. Disease resistance in the transgenic plants was correlated with the *ChiC* expression levels (Itoh et al., 2003).

Three genes, *ech42*, *nag70* and *gluc78*, encoding hydrolytic enzymes, from a biocontrol fungus *Trichoderma atroviride*, were introduced in single or in combinations into rice. *Gluc78*-overexpressing transgenic

plants showed enhanced resistance to *M. grisea*, while transgenic plants over expressing the *ech42* gene encoding for an endochitinase increased resistance to *R. solani*, resulting in a reduction of 62% in the sheath blight disease index (Liu et al., 2004; Shah et al., 2008). The *ech42* expression level and chitinase activity were correlated with disease resistance (Liu et al., 2004; Shah et al., 2008). Nevertheless, exochitinase enhanced the effect of endochitinase on disease resistance in transgenic plants co-expressing *ech42* and *nag70* (Liu et al., 2004).

Microbial antimicrobial proteins

Several types of antimicrobial proteins identified from both plant origin and microbial origin have been exploited for their validities and utilities in molecular improvement of rice resistance against fungal and bacterial diseases. Defensins are small peptides (45 - 54 amino acids) and share common characters among plants, insects and mammals. Dm-AMP1, a defensin from *Dahlia merckii*, was introduced into rice. Expression levels of *Dm-AMP1* ranged from 0.43 to 0.57% of total soluble protein in transgenic plants. The plants expressing *Dm-AMP1* showed significantly improved resistance to *M. oryzae* and *R. solani*, by 84 and 72%, respectively. Interestingly, transgenic expression of Dm-AMP1 was not accompanied by an induction of PR gene expression (Jha et al., 2008). Similarly, transgenic rice overexpressing the wasabi defensin or *Mirabilis jalapa* antimicrobial protein *Mj-AMP2* gene exhibited resistance to rice blast (Kanzaki et al., 2002; Prasad et al., 2008). The average size of disease lesions of the transgenic plants was reduced to about half of that of non-transgenic plants (Kanzaki et al., 2002). Thus, expression of defensin in transgenic rice has the potential to provide broad-spectrum disease resistance (field resistance) against fungal pathogens.

The *Aspergillus giganteus* antifungal protein (AFP) has been reported to possess *in vitro* antifungal activity against various economically important fungal pathogens including *M. grisea*. Transgenic rice constitutively expressing AFP protein showed inheritance of the transgene without any effect on plant morphology, growth and development (Coca et al., 2004; Moreno et al., 2005). The AFP protein prepared from leaves of transgenic plants exhibited biologically inhibitory activity on the *in vitro* growth of *M. grisea* and the transgenic plants showed enhanced resistance to the blast disease (Coca et al., 2004; Moreno et al., 2005).

Puroindolines are small proteins reported to have *in vitro* antimicrobial properties. Transgenic rice plants that constitutively express the wheat puroindoline genes *PinA* and/or *Pin*B throughout the plants were produced. Puroindolins in leaf extracts of the transgenic plants reduced *in vitro* growth of *M. grisea* and *R. solani* by 35 - 50%. Transgenic rice expressing *PinA* and/or *PinB* showed significantly increased resistance to *M. grisea* and *R. solani* (Krishnamurthy et al., 2001).

Cecropins are a family of antimicrobial peptides, which constitute an important key component of the immune response in insects. Plant codon-optimized synthetic constructs of cecropin A gene from the giant silk moth *Hyalophora cecropia* were introduced into rice. The Cecropin A-expressing transgenic rice plants accumulated biologically active cecropin A protein and exhibited resistance to rice blast without an induction of PR gene expression (Coca et al., 2006). Similarly, transgenic rice plants overexpressing cecropin B gene showed a significant reduction in development of lesions caused by *X. oryzae* pv. *oryzae* (Sharma et al., 2000; Coca et al., 2004).

Generally, in most cases, constitutive expression of antimicrobial proteins in transgenic rice confers partial or moderate but not absolute resistance against pathogens.

Broad-spectrum resistance

The so-called “broad-spectrum resistance” is generally considered at two different levels, e.g. resistance to the majority of geographically different isolates of the same pathogen and resistance to two or more unrelated pathogens. Some of the cloned rice *R* genes have been found to confer broad-spectrum resistance against different races of a given pathogen and thus can be used in a traditional breeding program or transferred into elite rice varieties through transgenic approaches. One of the effective strategies for broad-spectrum plant resistance has been to exploit the signaling network that regulates the innate defense mechanisms against pathogen infection (Jones and Dangl, 2006). Functional genes or proteins of both plant origin and non-plant origin that positively regulates the signaling of systemic acquired resistance, which provides broad-spectrum resistance against viruses, bacteria, and fungi, will be useful sources for genetic engineering of broad-spectrum resistance in rice against multiple types of pathogens.

Defense signaling genes

Recent studies have showed that salicylic acid (SA)- and ethylene (ET)/jasmonic acid (JA)-mediated signaling pathways, which are critical to activation of defense responses of dicot plants against biotrophic and necrotrophic pathogens, respectively, also play important roles in rice disease resistance responses (Glazebrook, 2005). In rice, distinct mechanisms might be required for its defense responses against different pathogens because different types of defense-responsive genes were found to be involved in resistance to bacterial blight and fungal blast diseases (Wen et al., 2003; Ahn et al., 2005). However, it was also found that the SA- and ET/JA-mediated signaling pathways in rice may operate in concert and share some common components or biochemical events

(Qiu et al., 2007; Ding et al., 2008). NPR1 is a key regulator in the SA-mediated signaling pathway in Arabidopsis. Transgenic rice plants expressing *AtNPR1* showed enhanced disease resistance against *M. grisea* and *X. oryzae* pv. *oryzae* by priming the expression of SA-responsive endogenous genes, such as the *PR1b*, *PR5*, *PR10* and *PBZ1* (Chern et al., 2001; Fitzgerald et al., 2004; Quilis et al., 2008). The rice genome contains five *NR1*-like genes and three of them, *OsNPR1*, *OsNPR2* and *OsNPR3*, were induced by infection of *X oryzae* pv. *oryzae* and *M. grisea. OsNPR1* is the rice orthologue of Arabidopsis *NPR1*. Overexpression of *OsNPR1* conferred disease resistance to bacterial blight, but also enhanced herbivore susceptibility in transgenic rice plants (Chern et al., 2005; Yuan et al., 2007). OsNPR1 might mediate antagonistic cross-talk between the SA- and JA-dependent pathways and thus provides a practical approach for engineering of broad-spectrum disease resistance in rice (Yuan et al., 2007). Constitutive expression of pathogen-inducible genes encoding transcriptional factors enhances disease resistance against *M. grisea* through activation of expression of many defense-related genes (Zhang et al., 2008; Wang et al., 2008). On other hand, genetic manipulation of the JA biosynthesis pathway has also been shown to improve rice disease resistance against fungal and bacterial pathogens. It was found that transgenic rice plants overexpressing a pathogen-inducible *OsAOS2* gene, which encodes an allene oxide synthase, a key enzyme in the JA biosynthetic pathway, accumulated higher levels of JA, up-regulated expression of PR genes and increased resistance to *M. grisea* infection (Mei et al., 2006). Genetic modification of JA-related fatty acid metabolism by suppression of β-3 fatty acid desaturases, allene oxide cyclase or 12-oxo-phytodienoic acid reductase also increased disease resistance against *M. grisea* (Yara et al., 2007, 2008a, b).

Reactive oxygen species

Oxidative burst, mediated by hydrogen peroxide (H_2O_2), has been recognized as a key component of the plant defense. Glucose oxidase (GOX), an enzyme occurring in some bacteria and fungi, brings about the oxidation of β-D-glucose, yielding gluconic acid and H_2O_2. Transgenic rice plants with constitutive and pathogen-induced expression of an *Aspergillus niger GOX* gene lead to increases in the endogenous levels of H_2O_2, which in turn caused typical cell death and activated the expression of several defense genes. The GOX-over expressing transgenic rice plants showed enhanced resistance to both *M. grisea* and *X. oryzae* pv. *oryzae* (Kachroo et al., 2003). However, it should be done with caution since high levels of H_2O_2 in transgenic plants may cause metabolic disturbances interfering with normal growth and development. Using pathogen-inducible expression of H_2O_2-generating genes may be an effective way to confer broad-spectrum resistance in rice without the penalty of causing any developmental abnormalities or metabolic errors.

Microbe-derived elicitor genes

One promising approach to the achievement of broad-spectrum resistance is to incorporate genes that elicit general defense responses into rice. Recently, several microbial proteinaceous elicitors have been shown to induce systemic acquired resistance in plants by the activation of both SA- and ET/JA-mediated signaling pathways. The bacterial harpin and flagellin are two examples that have been studied for the possibility in genetic engineering of broad-spectrum resistance in rice. Recently, Shao et al. (2008) introduced a harpin-encoding gene *hrf1*, derived from *X. oryzae* pv. *oryzae*, into rice and generated transgenic rice lines with overexpression of the *hrf1* gene. Disease assays revealed that the *hrf1*-overexpressing transgenic rice plants and their T1-T7 progenies were highly resistant to all major *M. grisea* races in rice-growing areas. Defense responses including enhanced expression of PR genes and increased content of silicon in leaves were activated in the *hrf1*-overexpressing transgenic plants, and the formation of melanized appressoria was inhibited on leaves of the transgenic plants (Shao et al., 2008). This study suggests that harpins of phytopathogenic bacteria may offer new opportunities for generating broad-spectrum resistance in rice. On the other hand, flagellin is a component of bacterial flagella and acts as a proteinaceous elicitor of defense responses in plants. The flagellin gene from a phytopathogenic bacterium, *Acidovorax avenae* strain N1141, was introduced into rice and the resultant transgenic rice plants accumulated flagellin at various levels. The transgenic plants showed increased expression of defense genes, H_2O_2 production and cell death, suggesting that the flagellin triggers innate immune responses in the transgenic rice. When inoculated with *M. grisea*, the transgenic plants exhibited enhanced resistance, suggesting that the flagellin approach might provide a new strategy for developing genetically engineered disease-resistant rice varieties (Takakura et al., 2008).

Pyramiding resistance

The resistance in newly released varieties can be lost quickly due to the high level of instability in the pathogen population. One way to circumvent this problem is to develop genetic engineered rice varieties with (i) a combination of genes encoding/controlling interdependent or synergistic subcomponents of the disease-resistant trait to realize effective resistance against a particular disease or (ii) a combination of genes associated with different diseases to realize a broad-spectrum resistance. Pyra-

miding *Xa21* gene, a chitinase gene, and a Bt-fusion gene into IR72 through conventional crossing of two independent transgenic homozygous rice lines confers multiple resistances against *X. oryzae* pv. *oryzae*, *R. solani* and yellow stem borer (Datta et al., 2002). Combination of marker-assisted breeding and genetic transformation yielded rice lines resistant to blast and leaf blight diseases by pyramiding *Pi1*, *Piz5* (major blast resistance genes), and *Xa21* (Narayanan et al., 2004). Genetic engineering of rice to pyramid a maize ribosome-inactivating protein gene and a rice basic chitinase gene confers enhanced resistance of the transgenic rice lines against three fungal pathogens, *R. solani*, *Bipolaris oryzae*, and *M. grisea* (Kim et al., 2003). Co-expression of rice chitinase and thaumatin-like protein in an elite indica rice line resulted in significant higher level of resistance against *R. solani* (Kalpana et al., 2006). Transgenic plants pyramided with *chi11*, *tlp* and *Xa21* showed an enhanced resistance to both sheath blight and bacterial blight diseases (Maruthasalam et al., 2007). Combined expression of chitinase and β-1,3-glucanase genes in indica rice enhances resistance against *R. solani* (Sridevi et al., 2008), while transgenic lines expressing four antifungal genes including *RCH10*, *RAC22*, β-*Glu* and *B-RIP* showed not only high resistance to *M. grisea* but also enhanced resistance to rice false smut (*Ustilaginoidea virens*) and rice kernel smut (*Tilletia barclayana*) (Zhu et al., 2007). Therefore, an ingeniously planned genetic engineering involving a well-balanced expression of transgenes with different modes of action would ensure broad-spectrum and durable resistance against pathogens.

FUTURE DIRECTION AND STRATEGIES ON GENETIC ENGINEERING OF RICE DISEASE RESISTANCE

Our knowledge of molecular events occurring during rice-pathogen interactions has expanded significantly in the last decade and, based on this knowledge, several strategies have emerged for developing rice varieties resistant to pathogens. In the cases where manipulation of resistance is achieved by expression of a single or multiple antimicrobial proteins or phytoalexins, the engineered resistance in transgenic rice is not absolute and most of them only show partial or moderate resistance. On the other hand, a number of disease resistance genes have been isolated from rice and some of them have been shown to confer broad-spectrum resistance against several races of pathogens. However, the high level of specificity of resistance conferred by most of the cloned *R* genes restricts their future use in developing broad-spectrum resistance by the means of genetic engineering since this kind of specific resistance in the genetically engineered rice will be easily overcome due to changes in the pathogen population.

Engineering of varieties with durable and broad-spectrum resistance is an ultimate goal for breeding and improving rice resistance against multiple diseases. This will be achieved probably through genetic manipulation of the regulatory mechanisms and signaling processes controlling the coordinate activation of multiple defense responses. Extensive studies of rice disease resistance responses using genomics and proteomics approaches will lead to identification of novel genes that are involved in the defense signaling pathways and subsequent metabolic pathways. These genes will be very useful in the generation of new rice varieties with high level of resistance (probably durable resistance) against multiple diseases caused by different types of pathogens. While exploiting the genes in signaling pathways for developing disease resistant transgenic rice, it must be noted that the defense signaling genes may also play roles in other pathways, resulting in undesirable side effects in transgenic plants. The position that the genes function in the pathway and expression of the transgene in a correct temporal and spatial pattern will be of critical importance.

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