environment. Applications can be reviewed by the public for 30 days. This gives members of the public the opportunity to provide relevant information and express their opinions. RDA will give notice of the results of the inspection to applicants within 270 days of receiving the application. According to the guidelines, RDA established a special inspection committee. This committee consists of 15 members working in agriculture, plant breeding, entomology, molecular biology, ecology, and anti-GMO group.

As of June 2004, a total of 11 GMO events of 3 crops have been applied for the environmental safety approval. GMOs under being reviewed by the environment safety authority, the Rural Development Administration (RDA), are 1 herbicide tolerant soybean, 2 insect resistant maize, 3 herbicide tolerant maize, 1 herbicide/insect resistant maize, 1 herbicide tolerant cotton, and 3 insect resistant cotton. Among them, Roundup Ready Soybean(GTS-40-3-2) and insect resistant maize(Mon 810) of Monsanto have been officially cleared for their environmental safety on May 18, and on June 4, respectively by environmental safety authority. Besides the Monsanto Korea, Bayer Crop Science, and DuPont Korea are among the applicants for the environment safety approval. Information on approval status of GMO environment Risk Assessment is available at the RDA homepage of http://www2.rda.go.kr/gmo/english/e_index.asp.

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SIV-2

MAP kinase kinase kinase as a positive defense regulator in rice-blast fungus interactions

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Abstract

We have found the role of rice mitogen-activated protein kinase kinase kinase (MAPKKK), *OsEDR1*, as controlling hypersensitive response (HR) and increased disease resistance to rice blast fungus *Magnaporthe grisea*. Generation of transgenic rice plants through introduction of the over-expression construct of *OsEDR1* using *Agrobacterium*-mediated transformation results in lesion mimic phenotype. Up-regulation of defense mechanism was detected through detection of increased transcription level of rice *PBZ1* and *PR1a*. Inoculation of rice blast fungus on the lesion mimic transgenic lines displayed significantly increased resistance. The disease symptoms were arrested like HR responses which are commonly detected in the incompatible interactions. High accumulation of phenolic compounds around developing lesions was detected under UV light. There was variation among transgenic lines on the timing of lesion progression as well as the lesion numbers on the rice leaves. Transgenic lines with few lesions also show increased resistance as well as equal amount of grain yields compared to that of wild type rice cultivar Nipponbare. This is the first report of the MAPKKK as a positive regulator molecule on defense mechanism through inducing HR-like cell death lesion mimic phenotype. The application of *OsEDR1* is highly expected for the development of resistant cultivars against rice pathogens.

Introduction

Disease resistance mechanism of plant has been a major research area for many scientists in order to solve the yield loss problems by pathogens. Breeders have been trying to generate resistant cultivars through introgression of resistance genes which confer complete resistance into the customer most wanted, but susceptible cultivars. There have been many reports on cloning and functional analysis of *R*-genes for the purpose of using them for molecular breeding sources of various plants (*Dilbirligi* et al., 2004). But, as proved by the breeding programs so far, a simple introgression of *R*-genes into the susceptible cultivars for generating resistant one result in resistance breakdown through the appearance of new races infecting *R*-gene-mediated new resistant cultivars (Kiyosawa, 1982; Bonman et al., 1986). This unexpected disease occurrence brings the necessity of developing durable resistant cultivars.

There are two types of resistance which has different level of defense against disease. The one is field resistance which is manifested by synergistic effect of multi gene products having individual role in defense activities (Parlevliet, 1983). This has an advantage in durability, but unfortunately, it show partial resistance (Ezuka, 1972; Yeh and Bonman, 1986). The characteristics of partial resistance are slow disease development and less lesions, and that reduces the epidemic potential (Roumen, 1994). Because of the generic disadvantage of *R*-gene-mediated resistance, field resistance is regarded as an alternative method for

generating efficient resistant cultivar. This resistance shows non-race specificity in the resistance responses to various pathogens, which results in broad spectrum resistance.

The other resistance is true resistance which dominated by resistance (*R*) gene in the host plant (Flor, 1971). The recognition of avirulence (*avr*) gene product from pathogen by the *R*-gene results in the hypersensitive response (HR). HR is regarded as the most efficient and rapid defense mechanism for all the infections by various plant pathogens to halt disease development on its earliest stage even before spreading to the neighboring cells from the first infected cell (Hammond-Kosack and Jones 1996; Greenberg 1997, Lam et al. 2001). Rapid cell death significantly decreases viability of pathogens by reducing usable nutrients for pathogens inside the infected cells. But, it has been largely unknown about the involved signaling pathways leading to the two different resistance responses. Therefore, understanding the relatedness of the two resistance mechanisms has important implications in the prevention and management of many plant diseases and finding key regulators of defense mechanism. In addition, introducing them into the crop will be a good model for molecular breeding of resistant crops.

The MAPK cascade is a sequential phosphorylation pathway from MAPKKK-MAPKK-MAPK and underlying transcription factors (Xing et al., 2002). The MAPK cascades were regarded as parallel pathway or signal convergence and divergence center as positive and negative regulatory mechanisms (Xing et al., 2002). MAPK cascade has a major role in signal transduction pathway for leading to efficient defense responses in insects and animals (Xia et al., 1995; Zanke et al., 1996; Chen et al., 1996; Verheij et al., 1996). It transfer received signals from the Toll-like receptor (TLR)-mediated recognition down to the individual responses for efficient disease control. In plants, MAPK cascade has been shown to have roles as important signaling pathway of biotic and abiotic stresses (Tena et al., 2001; Hirt, 1997). Some MAPKs transcripts are known to accumulate very rapidly upon biotic and abiotic stresses and that suggest the important role of MAPK cascade in defense signal pathways (Cheong *et al.*, 2003; Xiong and Yang, 2003; Kim et al., 2003). Recently, the involvement of MAPK cascades in disease defense mechanism has been focused as important controlling modules as the evolutionarily conserved eukaryotic signaling pathways including plants and animals (MAPK group, 2002; Chang and Karin, 2001; Widmann et al., 1999). But, the detail molecular mechanisms after the recognition of the pathogen-derived signals are largely unknown in plants (Aderem and Ulevitch 2000; Khush and Lemaitre 2000). In contrast to animals, plant must have developed evolutionary sophisticated signaling pathways to survive during its life time in one place where there have been numerous biotic and abotic stresses (Dangle and Jones, 2001; Hammond-Kosack and Jones, 1996). There has been sufficient data that various plant pathogens activate common defense responses displayed as up-regulation of pathogenesis-related (PR) genes (Romeis et al., 1999; Blume et al., 2000; Hirt and Scheel, 2000; Zhang and Klessig, 2001). MAP kinase molecules are regarded as major candidates for signal transduction pathway in defense responses against plant pathogens (Romeis, 2001; Tena et al., 2000). In fact, the functions of a few MAP kinases were reported as defense response inducers on plant disease (Ligterink et al., 1997; Zhang and Klessig, 1998; Zhang et al., 1998; Nuhse et al., 2000; Cardinale et al., 2000; Lee et al., 2001; Song and Goodman, 2002; Xiong and Yang, 2003), but the detail identification of receptors and sequencially interacting MAPKKKs, MAPKKs and MAPKs and the downstream effector molecules after MAPK cascades are mostly unknown.

Here, we report rice MAPKKK, *OsEDR1* (Kim et al., 2003) which is an HR response regulator gene and it also increase defense reaction against rice blast fungus *M. grisea*. The functional analysis as well as molecular analysis of *OsEDR1* indicates that the gene might be an upstream defense signaling molecule in rice MAPK cascade leading to up-regulation of defense-related genes. Further characterization of the *OsEDR1*-mediated defense signal cascade will elucidate efficient defense response through HR responses.

Discussion

Host plants can recognize infection of pathogens earlier than direct gene-for-gene interaction occurs between protein products from host plant and pathogen. This early response implies that there must be very rapid onset of defense reactions upon attack of pathogen before penetration occurs into the host cells. Although there are few reports on direct interaction between *R*-genes and *avr*-genes (Leister and Katagiri, 2000; Scofield, 1996; Tang, 1996), the sequential signaling has not been clearly elucidated, yet. The defense signaling pathway in plant have been investigated for decades, but the information of the major molecular components from recognition of pathogens to the defense reactions have not been well characterized. In contrast, the MAPK cascade is known as the central controlling box on signal transduction pathway through serial phosphorylation of individual components leading to apoptosis which is an important defense reaction in mammalian system. This MAPK cascades are conserved both in yeast and mammalian system and it support the possibility of existence of similar MAPK cascade in plant as the general signaling network as eukaryotic organisms. In fact, activation of the JNK pathway, which is a MAPK pathway, has been shown to be a common phenomenon in apoptotic cell death in mammalian system (Xia et al., 1995; Zanke et al., 1996; Chen et al., 1996; Verheij et al., 1996). Several reports on the involvement of individual MAPK in defense responses have strongly suggest the possible role of MAPK-mediated defense signaling pathway in plants.

Recently, *OsMAPK5* has been proved to do an important role in resistance against rice blast fungal pathogen *M. grisea*. (Xiong and Yang, 2003). But, the defense-related MAPK cascade from the unknown upstream molecule MAPKKK to *OsMAPK5* has not been understood. The identification of MAPKKK as the starting molecule in serial phosphorylations is important step because there is a hypothesis that the MAPKKK delivers signals through different MAPK-mediated pathways to induce individual responses (Xing et al., 2002). The transcription level of *OsEDR1* mRNA from all the over-expression transgenic lines were not so clear by the Northern hybridization. The constitutive low expression level of *OsEDR1* mRNA can be estimated in two different ways. The first reason is because *OsEDR1* was expressed under the control of strong 35S promoter which is recommended

one in dicot, not in monocot plants. The second reason is that the up-regulation induces rapid cell death and eventually death of whole plant which is a typical lethal phenotype. That is because still the detected transcription level of *OsEDR1* in over-expression lines was far below of the 35S promoter expression level of other case of transgenic lines and, that indicates of intolerance of a high level of expression of *OsEDR1* for survival of the organism. The similar cell death phenomenon is reported in mammalian system that over-expression of MEKK1 which is a mammalian MAPKKK, induced apoptosis. (Xia et al., 1995) and the result support the possibility that *OsEDR1* may have the similar regulatory function of apoptosis in rice.

The HR reaction has been characterized as rapid cell death and regarded as the major defense responses against attack by the pathogens. There were several reports on lesion mimic mutants showing resistance to various pathogens. The *OsEDR1*-mediated lesion mimic phenotype shows typical positive sign of up-regulation of defense mechanism. There was up-regulation of two representative PR-genes, *PR-1a* and *PR-10* of rice, and that represents the acquired resistance in the transgenic lines. (Xiong and Yang, 2003).

The low concentration of localization of OsEDR1::GFP chimeric protein implies that the expression level in onion cell is far lower than that by the control GFP vector. Those results strongly represent that the over-expression of OsEDR1::GFP induces cell death and only mild expressed OsEDR1::GFP chimeric proteins were remained visible in the live cells in the nuclei. The nuclei targeting of OsEDR1 protein represent its role as a transcriptional regulator as complex with transcription factors leading to cell death. There was a report on the nuclear targeting of MAPK (Ligterink et al., 1997), but this is the first report of nuclear localization of MAPKKK. The role of OsEDR1 in the nuclei needs to be elucidated. Many empty cells were detected only in the OsEDR1::GFP-bombarded onion cells, but most of the cells were normal in GFP vector-bombarded onion cells.

Activation of defense mechanism inevitably decreases crop yields by constitutive consumption of energy doing up-regulation of many defense related genes. In some case of lesion mimic mutant show expected significant yield loss. Thus, generation of resistant cultivars without any yield loss is highly recommended for both classical and molecular breeding methods. Some *OsEDR1* over-expression lines produce similar yields like that from control Nipponbare, and that means the *OsEDR1*-induced molecular breeding is an appropriate method of generation of resistant cultivar.

Phytoalexins and phenolic compounds are observed in plant cell undergoing HR (Hammond-Kosack et al. 1996) as well as in the spontaneous lesions of some lesion mimic mutants (Greenberg et al. 1994; Wolter et al. 1993; Becker et al. 1993). Experiments with susceptible and resistant rice cultivars suggest that sakuranetin, momilactone A and oryzalexin S accumulate in sufficient concentrations to provide such resistance (Dillon et al. 1997). Thus, it is tempting to postulate that the materials were a reason for enhanced resistance of *OsEDR1* transgenic lines showing accumulation of phenolic compounds qkto rice pathogens.

The rapid accumulation of phytoalexin around the infection sites is an efficient way of defeating colonization or spreading of the pathogens in the host cells. Lesion specific accumulation of phenolic compounds in *OsEDR1* over-expression lines is also strong evidence of the occurrence of resistance through direct interaction with pathogens as well as HR reactions triggering defense responses.

MAPK cascade is very complicated network of delivering signals from diverse receptors. Thus, identification of pathogen receptors as well as series of downstream molecules leading to individual defense responses is required to understand defense signal pathway. Constitutive *OsEDR1* expression over the appropriate level induces too much cell death and the death of the whole plant, instead of optimal upregulation of defense mechanisms. Thus regulating expression level below the optimal level only at the required tissues on time when the pathogens infects is highly recommended direction for molecular breeding of resistant cultivar using HR-mediated regulatory genes. We are currently developing promoter system for the efficient regulation of the positive regulatory gene *OsEDR1* for generation of resistant cultivars for practical purposes.

This is the first report on MAPKKK as the positive regulator on cell death mechanism inducing defense responses. This data strongly suggest that the conservation of defense-related MAPK signaling pathway in plants just like those in yeast and mammalian systems.

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SIV-3

How the plant disease resistance genes can continue to recognize pathogen's infection.

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Most of plant R-gene's function is based on its recognizing ability, directly or indirectly, of the pathogen's corresponding avirulence gene products. Not to be overcome by the rapid mutation of pathogen's genome, plant R-genes also need to have some diversification mechanism, in addition to its maintenance mechanism in population. In order to analyze the mechanism of plant R-genes diversification, we have compared the base changes in the neighborhood region of rather isolated resistance genes, between the resistant and susceptible cultivars in rice and then in *Arabidopsis*. At the first search on the rice blast resistance gene *Pi-b*, the mutation rate was found to be more than ten times higher than the average of the genome, in about 60-80 kb region around the gene. This high-variability was not confined within the R-gene, of which LRR domain, the specificity determining factor, may have been subject to some diversification selection. Major types of the mobilization were accumulation of less than a few bases of small substitutions and insertion-and-deletions (indels). Density of small transposable elements like mites was about the same level as the other regions.

This tendency of high genome mobilization around R genes or its analogues was confirmed in *Arabidopsis* too, by comparing their sequences between ecotypes Columbia and Landsberg. Analysis about 15 kb around some rather isolated R gene and analogues revealed that the variability of the genome in this region is about 5 times higher than the average of the total genome, although there are variatons in the extent of this variability, from the normal level to more than ten times of that. Hypothesis that the mutations of *Arabidopsis* are explained as biallelic origins was refuted for R-genes that are experiencing high rate of genome mobilization.

To further characterize the R-gene analogue in the genome, more than 1000 R-gene analogues (RGA) were fine mapped in the rice genome by a combination of HEGS (High Efficiency Genome Scanning) system and RGA-scanning, both of them were developed by us. These systems can be readily applied for any other crops irrespective of the presence of genome sequence database.

SIV-4

Molecular marker assisted breeding for blast resistance in rice

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Rice blast disease caused by the fungal pathogen *Magnaporthe grisea* is one of the most serious and widespread diseases of rice worldwide. The deployment of resistant cultivars is the most effective and economical way of controlling blast disease, so breeding for resistant cultivars continues to be a priority in rice improvement (Kim *et al.* 1994). Comprehensive genetic studies on blast resistance have been conducted. At least 20 dominant genes conferring complete resistance to rice blast and 10 quantitative trait loci associated with partial resistance have been identified and several have been located via linkage to molecular markers (McCouch *et al.* 1994) and two of them, *Pi-b* and *Pi-ta*, have been cloned (Wang *et al.* 1999, Bryan *et al.* 2000).

Ilpumbyeo is an elite line widely cultivated in Korea. However, this cultivar has become increasing susceptible to blast, resulting in a decline of the area planted to this cultivar. The objectives of this study were to improve the blast resistance of Ilpumbyeo by introgressing the blast resistance gene(s) of *Moroberekan* by molecular marker-assisted selection in the backcrossing process; and to evaluate the effects of such improvement on the agronomic performance of Ilpumbyeo and the