Hetrotrimetric G proteins transmit signals for regulating deveopment and virulence

We isolated three putative Ga subunits, designated as *FgGPA1*, *FgGPA2* and *FgGPA3*, respectively. Deletion of each Ga subunit affected pathogenecity, sexual development, and secondary metabolism such as mycotoxin production and pigmentation. The *FgGPA1* is homologous to the *fadA* gene in the model fungus *Aspergillus nidulans*, and to the *magB* in the rice blast fungus *Magnaporthe grisea*. The *FgGPA1*deletion mutant could not make fruiting body, leading to female sterility. Deletion of *FgGPA2*, which encodes *GanB* homolog of *A. nidulans*, resulted in normal perithecia formation but altered pigmentation and irregular colony margin. Furthermore, *FgGPA2* deletion mutant failed to cause disease on barley, indicating that *FgGPA2* plays an important role for pathogenicity. *FgGPA3* deletion resulted in decreased aerial hyphae, while perithecia formation seems to be greater than wild type, suggesting that the *FgGPA3* involves in activating hyphal growth but repressing sexual development.

Toxin biosynthesis genes involved in virulence.

We cloned and sequenced a *Tri13* homologs from DON and NIV chemotypes. Unlike the *Tri13* ORF of NIV chemotype, that of DON carried several mutations. To confirm the roles of the *Tri13* gene in trichothecene production, we altered toxin production in the two chemotypes by gene manipulation. Targeted deletion of *Tri13* from the genome of NIV chemotype caused DON production rather than NIV. Heterologous expression of the NIV chemotype *Tri13* gene conferred on DON chemotype the ability to synthesize NIV. The functional analysis of the *Tri13* gene provides the first clear evidence for genetic basis of the DON and NIV chemotypes in *G. zeae*. We also isolated genes encoding a type I polyketide synthase (PKS) and a putative laccase, all of which are required for aurofusarin biosynthesis. While screening insertional mutants of *G. zeae* generated by REMI procedure, we selected a mutant (S4B3076) that was unable to produce aurofusarin. Genomic DNA region of the vector insertion site [a *Hin*dIII site 38 bp upstream the ORF encoding a putative laccase, designated *Gip1*] in S4B3076 was identified as contig 1.116 in the *F. graminearum* genome databases. On a 30 kb region of the insertion site, close to *Gip1*, are 10 more ORFs including putative transcription regulators and an ORF (identified as *PKS12*), showing high similarity to the type I fungal PKS genes involved in pigment biosynthesis. Targeted gene deletion analysis confirmed that both *Gip1* and *PKS12* were responsible for aurofusarin production by *G. zeae*.

Conclusion

We have screened 20,000 REMI transformants, and 115 strains have been selected as mutants defective for virulence, sexual development, or mycotoxin production. We have determined the functions of 13 genes responsible for disease occurrence. The candidate genes for molecular breeding against the head blight fungus could be trichothecene-biosynthesis genes. Trichothecenes are known to be involved in virulence. Among the *Tri* genes, *Tri11* and *Tri12* are efflux pump gene and immunity gene, respectively. Transgenic rice resistant to head blight could be achieved by transforming these genes into rice.

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SV-3

Investigation of the molecular mechanisms involved in the resistance response during ripening stages of nonclimacteric pepper fruit against *Colletotrichum gloeosporioides*

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Fruit ripening represents a genetically synchronized system that involves developmental process unique to plant species (Giovannoni, 1993). The feature that ethylene hastens ripening distinguishes climacteric fruit such as apple and tomato from nonclimacteric fruit such as pineapple and strawberry, in which the progress of ripening appears to be independent of ethylene. Ripe fruits generally exhibit increased susceptibility to pathogen infection (Swinburn, 1983). However, in several nonclimacteric

fruits such as cherry, grape, and pepper, resistance against phytopathogens increases during ripening (Fils-Lycaon et al., 1996; Robinson et al., 1997; Oh et al., 1998; Salzman et al., 1998). These studies suggest that fruits as a reproductive organ have their own protection mechanism against pathogens to maintain their integrity during seed maturation.

Colletotrichum gloeosporioides (Penz.) causes anthracnose disease in fruit crops (Daykin, 1984; Dodd et al., 1991) such as pepper (*Capsicum annuum* L.) (Kim et al., 1986; Manandhar et al., 1995). We have established that *C. gloeosporioides* has susceptible and resistant interactions with pepper fruits during pre- and post-ripening stages, respectively (Oh et al., 1998; Kim et al., 1999; Oh et al., 1999a). In this pathosystem, higher levels of appressorium and infection hypha formation were observed on the unripe fruit than on the ripe fruit at 12 h and 24 h after inoculation (HAI), respectively. After that, initial anthracnose symptoms were detected only on the unripe fruit after 48 HAI, and typical sunken necrosis occurred within 120 HAI. We have been interested in elucidating a molecular mechanism by which resistance is induced against fungal infection during ripening of nonclimacteric pepper fruit.

To investigate the molecular mechanism involved in the incompatible interaction (Oh et al., 1999b, c; Oh et al., 2003), we isolated genes differentially expressed in the ripe fruit, but not in the unripe fruit upon fungal infection using mRNA differential display. Several full-length cDNA clones such as pepper defensin *j1-1, PepThi, PepCYP, PepEST,* and *PepTLP* were isolated and characterized.

The *PepTLP* gene encodes a protein homologous to other thaumatin-like proteins and contains 16 conserved cysteine residues and the consensus pattern of thaumatin. The *PepTLP* gene expression is developmentally regulated during ripening. The accumulation of *PepTLP* mRNA and PepTLP protein in the incompatible interaction was higher than that in the compatible one. The expression of the *PepTLP* gene upon fungal infection was a rise from the early-breaker fruit. The development of anthracnose became significantly prevented with beginning of fruit ripening, and the sum total of sugar accumulation increased. The results suggest that the *PepTLP* gene can be used as a molecular maker in probing for disease resistance, ripening, and sugar accumulation in the nonclimacteric pepper fruits.

The defensin gene, *j1-1*, and the thionin-like gene, *PepThi*, were developmentally regulated during fruit ripening, organ-specifically regulated, and differentially induced during the compatible and incompatible interactions. The fungal-inducible *PepThi* gene is highly inducible only in the unripe fruit by salicylic acid. In both ripe and unripe fruits, it was induced by wounding, but not by jasmonic acid. The expression of *j1-1* gene is enhanced in the unripe fruit by jasmonic acid, while suppressed in the ripe fruit. These results suggest that both small and cysteine-rich protein genes are induced via different signal transduction pathways during fruit ripening to protect the reproductive organs against biotic and abiotic stresses. We have generated over-expression of *j1-1* transgenic *Arabidopsis* and tobacco plants and examined whether the transgenic plants show disease resistance against fugal infection.

Deduced amino acid sequence of the *PepEST* cDNA showed homology to both esterases and lipases. Inhibition of PepEST activity by a specific inhibitor of serine hydrolase demonstrated that a serine residue is critical for the enzyme activity. Expression of *PepEST* gene was fruit-specific in response to *C. gloeosporioides* infection, and up-regulated by wounding or jasmonic acid treatment during ripening. Immunochemical examination revealed that PepEST accumulation was localized in epidermal and cortical cell layers in infected ripe fruit, but rarely even in epidermal cells in infected unripe one. Over-expression of *PepEST* in transgenic *Arabidopsis* plants caused restriction of *Alternaria brassicicola* colonization by inhibition of spore production and constitutive up-regulation of *PR-2* and *PR-4* gene. These results suggest that *PepEST* is involved in the resistance of ripe fruit to *C. gloeosporioides* infection.

In order to further investigate esterase function in plants, we isolated and characterized two esterases in *Arabidopsis* plants. The recombinant protein of AtEST1 showed specific enzyme activity for *p*-nitrophenyl butyrate *in vitro*. A knockout mutant *atest1* that selected by mutant screening showed enhance susceptibility to *Botrytis cineria*. In addition, over-expression of *AtEST1* gene in transgenic *Arabidopsis* plants showed enhanced disease resistance against *B. cineria*. These results suggest that esterase plays a critical role for defense against fugal infection in plants.

Taken together, to protect ripe fruit against *C. gloeosporioides* infection, the fruit may deploy at least two different defense mechanisms: one induced by fungal elicitors before fungal appressorium formation, and the other induced by plant-derived signals generated after fungal penetration.

We have used cDNA microarray technology to study about genes expressed during ripening. The cDNA clones that show more than three fold difference in response to fruit ripening were mainly related to plant defense and environmental stress. In addition, genes involved in transcription, secondary metabolism, cell wall metabolism, and signal transduction were also identified. This study showed the relevance of microarray technology in identifying many genes involved in resistance of pepper fruit during ripening.

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SV-4

Enhancing durable resistance of rice cultivars to Korean rice blast fungus

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1) Breakdown of blast resistance and change of *Magnaporthe grisea* population structure in Korea

Host resistance, for its economical benefits, has been used most extensively as a tool for crop disease management. Extensive pathogenic differentiation of rice blast fungus, *Magnaporthe grisea,* has been reported in many countries resulting in the breakdown of resistant cultivars of rice. Therefore, selection of resistance source and deployment of resistance genes should be based on the pathotypic structure of pathogen populations in the field. Most of the Korean commercial cultivars planted during the early 1960s were regarded to have the resistance gene, *Pi-a,* for blast. Since severe incidence of blast had been frequently observed, a new resistant variety Kwanok possessing R gene *Pi-k* from Kanto 55 was released into farmers' field in 1966. However, the variety was unfortunately severely infected in 1969 by a new race at the panicle stage.

A semi-dwarf gene from indica type rice, TN1, was introduced into japonica rice Yukara in 1965. Since then, indica rices have been extensively used to develop Tongil-type varieties by crossing with japonica lines. There have been no severe blast epidemics during the 7 years since 1970 when Tongil-type varieties were released to farmers. However, panicle blast occurred regionally on rice cultivars, Tongil and Yushin in Jeonbug province in 1976. Severe blast epidemics were observed in all provinces in 1977 and 1978. Breakdown of resistance of Tongil-type varieties was due to the pathogenic differentiation, change of population structure and favorable environmental conditions.

Resistant cultivars, such as Youngpungbyeo and Gayabyeo, became susceptible to the new pathogenic races KI-315a and KI-315b in 1983. Race KI-401 was a newly identified race which could infect resistant cultivars Seonambyeo and Cheonmabyeo. There have been no resistant cultivars showing overall resistance to all the races distributed in the field since 1983. The race KI-409 was first identified from Namyangbyeo in 1985 and has been rapidly built up since 1990. Most commercial cultivars having *BL1* and *BL7* pedigrees, Jinmibyeo and Ilpumbyeo were susceptible to race the KI-409. The race KI-409 was isolated from 47 rice varieties and became a predominant race with 23.7% of distribution ratio in 1995.

The cultivars Daesan, Ilmi and Dongan-byeo have shown wide spectrum of resistance to many races including KJ-301, however, their resistance were broken-down by new races such as KI-1117a, KI-1113a and KJ-105a in 1999 and 2000.

2) *Virulence spectrum of Korean M. grisea*

Twenty-seven monogenic rice lines harboring major resistant gene for blast were screened to analyze their resistance spectrum to Korean blast fungus population using 190 isolates collected from 1985 to 2002. Especially, the monogenic line containing *Pi-9* gene was screened using 320 isolates. Based on the monogenic lines-blast isolate interactions, the 27 rice lines were classified into 9 groups. The Chinese rice cultivar LTH showed susceptible to all the tested isolates. These lines IRBLz-Fu, IRBL5-M and IRBL9-