

学位論文要旨 Dissertation Abstract

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学位論文題目 : **Molecular biological studies on probenazole inducible rice genes: *OsAP77* and *OsHAP2E*** (プロベナゾール誘導性イネ遺伝子に関する分子生物学的研究 : *OsAP77*及び*OsHAP2E*)
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Rice *aspartic protease* (*OsAP77*) and *heme activator protein* (*OsHAP2E*) genes have been characterized which are induced by probenazole (PBZ), a chemical inducer of disease resistance. To elucidate roles of *OsAP77* and *OsHAP2E* genes, the chimeric genes (*OsAP::GUS* and *OsHAP::GUS*) have been constructed to carry the structural gene encoding β -glucuronidase (GUS) driven by the promoter from *OsAP77* and *OsHAP2E*, respectively. These constructs were introduced into rice. Transgenic lines were tested for GUS staining. Only the wounded and surrounding tissues were stained blue for *OsAP::GUS* but not for *OsHAP::GUS*. However, when the chimeric gene (*OsHAPin::GUS*) was constructed to carry the *OsHAP77* promoter and its first intron, the transgenic lines of *OsHAPin::GUS* showed high GUS activity in the wounded and surrounding tissues. Thus these promoters responded to wounding. The transgenic lines were further examined for pathogen infection and some signaling molecules. When immersed in a solution containing salicylic acid, isonicotinic acid, abscisic acid or hydrogen peroxide, the GUS activity was observed exclusively in vascular tissues for *OsAP::GUS*, but in vascular tissues and mesophyll cells for *OsHAPin::GUS*. When inoculated with *Magnaporthe oryzae*, *Xanthomonas oryzae* pv. *oryzae* or *Cucumber mosaic virus* (CMV), the induced GUS activity was observed. The knockout mutant line of *OsAP77* by the insertion of retrotransposon *Tos17* was inoculated with *M. oryzae*, *X. oryzae* pv. *oryzae* or CMV, it showed an enhanced susceptibility. The transgenic rice line over-expressing *OsHAP2E* (*OsHAP2E-OX*) were examined for pathogen infections. When inoculated with *M. oryzae* or *X. oryzae* pv. *oryzae*, the lesion size was remarkably smaller in the line than in non-transgenic line. These results collectively suggest that *OsAP77* and *OsHAP2E* are involved in defense response against biotic stress. *OsHAP2E-OX* showed a lower amount of CMV and *Rice necrosis mosaic virus* (RNMV) RNAs accumulated compared with that in non-transgenic control plants. The *OsHAP2E-OX* lines showed no significant symptoms by RNMV while the control plants showed yellowing and

stunting. These results suggested that *OsHAP2E* is induced by virus infection and contributes to resistance against viral pathogens. These results collectively suggest that *OsAP77* and *OsHAP2E* are involved in defense response against biotic stress. Moreover, the over-expressed line of *OsHAP2E* showed resistance against salinity and drought and increased photosynthesis and tiller numbers; suggesting multiple roles in plants.