バイオサイエンス研究科　博士論文要旨

所属 (主指導官)	植物分子遺伝学講座 (島本　功　教授)		
氏名	Utut W. Suharsono	提出	平成　14 年 1 月 8 日
題目	Role of the heterotrimeric G protein in defense response of rice		

要旨

Heterotimeric G proteins involved in various system of signal transduction in plants, such as defense response. Heterotrimeric G proteins consist of three subunits (α , β and γ). A number of studies using activators and inhibitors of heterotrimeric G protein plants have suggested that heterotrimeric G protein are involved in defense signaling. However, direct evidence for the involvement is still lacking. To gain insights into the role of the heterotrimeric G protein in defense response, we used rice *dwarf1* (*d1*) mutants lacking a single-copy G α gene and addressed its role in disease resistance.

Four *d1* mutants (CM248, CM723, CM 1232, CM1361) with Kinmaze (WT) background were used in this study. Northen blot analysis showed that very low level of *d1* transcript were present in the three alleles, CM 723, CM1232 , CM1361; and CM248 contained transcripts of two distinct size compared that wild type. Involvement of G protein α subunit in defense response was examined by infection of leaf sheaths with an incompatible race 031 of the rice blast fungus. In wild type more than 90% infected cells exhibited HR (hypersensitive response) and in all *d1* mutants reduced number of infected cells showed HR. Therefore, we examined whether G α mRNA in leaves was induced by an incompatible rice blast. Results showed that G α mRNA was induced at 24 hours after infection with an incompatible race 031. We next examined expression of defense related genes (*PR1, PBZ1*) in leaves after infection with an incompatible race 031. Activation of *PR* gene in leaves of the mutants was delayed for 24-48 hr relative to the wild type. These results clearly indicated that *d1* mutations caused reduced resistance to an incompatible of rice blast. To examine whether the heterotrimeric G protein also involved in defense responses to bacterial pathogens of rice, four *d1* mutants were infected with a compatible race of bacterial blight, *Xanthomonas oryzae* pv. *oryzae*,. Results of experiment indicated that in all *d1* mutants, resistance was reduced. Together, this results indicated that the heterotrimeric G protein plays an important role in disease resistance of rice.

We next examined whether heterotrimeric G protein is also involved in responses to sphingoipid elicitors. We first examined whether G α mRNA was induced by a sphingolipid elicitor in rice cell cultures. Results showed that G α mRNA was induced at 2 hours after sphingolipid elicitor treatment . We next measured the H_2O_2 levels in rice suspension cultures generated from embryo-derived calli of four *d1* mutants. At 2 and 4 hours after the elicitor treatment , an increase in H_2O_2 levels relative to those prior to the treatment was greatly diminished in each of four d1 cell cultures compared to those in the wild-type cell cultures. In wild type, *PBZ1* expression was first detected at 4 hours after the elicitor treatment, peaking at 6 hours and gradually decreasing

from 6 to 12 hours. In contrast, in the *d1* mutant cell cultures, *PBZ1* expression was not detected at any times examined. These results indicated that *PBZ1* expression in *d1* cell cultures was completely suppressed.

We next examined whether OsRac1 also involved in sphingolipid signaling. To test this, we examined H_2O_2 production and *PBZ1* expression after sphingolipid treatment in wild type rice cell cultures expressing the constitutively active OsRac1-G19V and dominant-negative OsRac1-T24N. Sphingolipid–induced H_2O_2 production was strongly enhanced in rice cultures expressing the constitutively active OsRac1, whereas it was completely suppressed in cell cultures expressing the dominant-negative OsRac1. Expression of *PBZ1* was greatly enhanced by the constitutively active OsRac1, while its expression was completely suppressed by the dominant-negative OsRac1. These results clearly indicate that the small GTPase OsRac1 is also an important intermediate in sphingolipid signaling in rice cell cultures.

To determine relative positions of the heterotrimeric G protein and OsRac1 in signaling pathway of rice, we generated transgenic lines derived from CM1361 mutant expressing the constitutively active OsRac1. We next examined H_2O_2 production and *PBZ1* expression induced by the sphingolipid elicitor in three independent transgenic cell cultures. Levels of H_2O_2 production in the transgenic cell cultures expressing the constitutively active OsRac1 were restored to a level close to that wild type cell culture expressing the constitutively active OsRac1. In contrast, strong *PBZ1* induction observed in wild type expressing the constitutively active OsRac1 was not detected in all three transgenic cell cultures examined although its expression was constitutively induced in the absence of the elicitor. Interestingly, induction of endogenous OsRac1 expression, which was detected by a gene-specific probe in the wild type cell cultures, was suppressed in the *d1* cell cultures.

To see if the introduction of the constitutively active OsRac1 restores disease resistance of *d1* mutants, we examined resistance to rice blast and bacterial blight in transgenic *d1* plants expressing the constitutively active OsRac1. The results of the experiments indicated that transgenic *d1* plants expressing the constitutively active OsRac1 were fully resistant to an incompatible rice blast. For bacterial blight infection, the transgenic *d1* plants were also resistant to bacterial blight.

We next examined whether *d1* mutations influence cell death caused by sphingolipid elicitor in rice cells culture. At the low sphingolipid concentration used for H_2O_2 production and *PBZ1* induction, we did not detect cell death in wild type or *d1* cell cultures. However, in transgenic wild type cell cultures expressing the constitutively active form OsRac1, we found clear induction of cell death. In contrast, in the transgenic d1 cell cultures expressing the constitutively active OsRac1, cell death induction was greatly reduced. These results indicate that the heterotrimeric G proteins plays an important role in cell detah induced by sphingolipid elicitors in the presence of OsRac1.

We next examined whether or not heterotrimeric G proteins were also involved in N-acetylchitooligosaccharide elicitor signaling. We measured H_2O_2 production and *PBZ1* expression in four *d1* mutants cell culture after treatment with N-acetylchitooligosaccharide elicitor. Results of the experiments indicated that there were no difference in levels of H_2O_2 production and *PBZ1* expression between the mutants and wild type. These results suggest the existence of different signaling pathways for sphingolipid and N-acetylchitooligosaccharide elicitors in cultures rice cells.

Results of this study indicated the role of the heterotrimeric G protein in disease resistance of rice. Thus, we proposed models for defense signaling of rice in which the heterotrimeric G protein functions upstream of the small GTPase OsRac1 in the early steps of signaling.

論文審査結果の要旨

申請者氏名　　Utut Widyastuti Suharsono

動物および植物において3量体Gタンパク質は受容体を介したさまざまな信号伝達に関与していることが知られている。植物の病原体に対する抵抗性反応も、植物の受容体が病原体由来のタンパク質などを認識することによって誘導されるため、以前から3量体Gタンパク質の耐病性信号伝達系への関与が示唆されてきた。実際、3量体Gタンパク質の活性化物質や阻害物質を用いた間接的な研究から3量体Gタンパク質が耐病性反応に関与していることが示唆されていたものの、直接的な証拠は得られていなかった。本論文では、3量体Gタンパク質αサブユニット（Gα)を欠損したイネ *dwalf1*(*d1*)変異体を用いることによって、3量体Gタンパク質が耐病性信号伝達へ関与することを直接的に証明しており、非常に意味のある研究成果である。

本論文においてイネ *d1* 変異体とイネいもち病菌を用いることにより、3量体Gタンパク質が抵抗性遺伝子に依存した過敏感反応に関与することについて調べている。過敏感反応は、植物の抵抗性遺伝子と病原体の非病原性遺伝子の相互作用によって誘導され、その相互作用は動物などで見られる一般的なリガンドと受容体の関係に似ている。*d1* 変異体では、この過敏感反応が抑制されており、3量体Gタンパク質が受容体を介した抵抗性の誘導に重要な役割を担っていることを示唆している。また、Gα遺伝子の発現が非病原性の病原体特異的に誘導されていることも、非常に興味深い。さらに、イネにおいて耐病性反応を誘導することが知られている2種類の異なるエリシター（セレブロシドとN-アセチルキトオリゴ糖）を用いて3量体Gタンパク質の信号伝達系の解析を行っている。その結果、*d1* 変異体ではセレブロシド処理によって誘導される抵抗性反応が抑制されること、Gα遺伝子の発現がセレブロシドにより誘導されることなどから3量体Gタンパク質がエリシターの信号伝達系でも機能していることを明らかにした。しかし、N-アセチルキトオリゴ糖の信号伝達への関与は見られず、一部の受容体を介した信号伝達系には3量体Gタンパク質が関与していないことを示している。また、これまで抵抗性反応の重要な信号伝達因子であることが知られている低分子量Gタンパク質OsRac1について、3量体Gタンパク質との関係について分子生物・遺伝学的な解析を行ったところ、3量体Gタンパク質は抵抗性信号伝達系上でOsRac1の上流に位置していることが明らかになった。さらに本論文中で行った一連の研究により、3量体Gタンパク質を介した信号伝達系のモデルを提唱しており、抵抗性信号伝達系を理解する上で非常に有用な知見を提供している。また、ここで得られた知見は耐病性育種など、応用を考える上でも非常に重要なものになるものと考えられる。

以上のように、本論文は3量体Gタンパク質の耐病性への関与を明らかにしたもので、学術上、応用上貢献するところが少なくない。よって審査委員一同は、本論文が博士（バイオサイエンス）の学位論文として価値あるものと認めた。