論 文 の 内 容 の 要 旨

生産・環境生物学専攻

平成16年度10月博士課程入学（進学）

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論文題目：　Studies on the roles of DWARF3 protein during germination and senescence in rice

（発芽、老化過程におけるイネDWARF3タンパク質の役割に関する研究）

Tillering dwarf mutants［*dwarf3*（*d3*), *d10*, *d14*, *d17* and *d27*］in rice, which reduced plant stature and increased tiller number were previously characterized. Among them, the *D3* gene and the *D10* gene were identified by map-based cloning and were shown to encode proteins orthologous to *Arabidopsis* MAX2/ORE9 and MAX4, respectively. The *Arabidopsis max2*/*ore9* mutant was originally isolated by screening for mutants that show a delay of senescence. Thus, I examined whether the rice *d3* mutant also delays leaf senescence or cell death as in the case of the *Arabidopsis max2*/*ore9* mutant. On the other hand, while investigating characteristics of the rice *d3* mutant, I noticed that the lengths of coleoptile and mesocotyl were significantly longer in *d3* than in its reference line, cv. Shiokari during germination under darkness. However, the coleoptile and mesocotyl growth under light conditions was comparable between *d3* and Shiokari. It was interesting for us to clarify the reason for difference of the habits of coleoptile and

mesocotyl grown under darkness or under light conditions between *d3* and Shiokari. The phenotype of longer coleoptile and mesocotyl in *d3* under darkness was similar to the phenotype observed in rice jasmonic acid（JA）-deficient mutant *cpm1*（*coleoptile photomorphogenesis1*）. Thus, I investigated whether the plant hormones such as JA were involved in the elongation of coleoptile and mesocotyl in *d3* under dark conditions.

1. The *d3* mutant has increased leaf longevity during senescence or cell death induced by darkness, H_2O_2 or jasmonic acid.

Senescence or cell death in plant leaves is known to be inducible by darkness, H_2O_2 and JA. When the *Arabidopsis* gene *MAX2/ORE9* is disrupted, leaf senescence or cell death in response to the above stimuli is delayed. Because the rice gene *D3* is orthologous to *MAX2/ORE9*, I wished to know whether disruption of *D3* also results in more longevity in leaves. I found that senescence or cell death induced by darkness, JA and H_2O_2 in the third leaf（as measured by chlorophyll degradation and membrane ion leakage）in *d3* rice mutant was delayed by 1-3 d compared to that in its reference line Shiokari. To confirm the delay of leaf senescence in *d3*, I examined the expression of three Senescence Associated Genes（SAGs; *Osl20*, *Osl85* and *Osl295*）that are known to be induced during leaf senescence. The mRNA levels of the three SAGs started to increase dramatically in Shiokari at 1 d after transfer to dark conditions, peaked at 3 d, and then decreased. However, in *d3*, induction of the SAGs was delayed for 1-3 d compared with the induction observed in Shiokari. Moreover, the mRNA levels of *D3*, *HTD1* and *D10*, which are orthologs of *Arabidopsis MAX2*/*ORE9*, *MAX3* and *MAX4*, respectively, increased during senescence or cell death. These results suggest that the induction of gene expression of the *D3*, *HTD1* and *D10* genes is associated with the occurrence of senescence or cell death in leaves. Together, it is suggested that D3 protein in rice, like MAX2/ORE9 in *Arabidopsis*, is involved in leaf longevity during leaf senescence or cell death induced by darkness, JA and H_2O_2.

2. Coleoptile and mesocotyl in the *d3* mutant are highly elongated under darkness, but not under light conditions.

When the *d3* mutant and Shiokari were germinated under darkness, I found that the lengths of coleoptile and mesocotyl were significantly longer in *d3* than in Shiokari. However, such differences of the coleoptile and mesocotyl lengths were not observed between the *d3* mutant and Shiokari seedlings grown under light conditions. In contrast, the lengths of coleoptiles grown under complete submergence（*i.e.*, anaerobic conditions）were comparable between *d3* and Shiokari even if the seedlings were germinated under darkness. Moreover, the timing of the stop of the coleoptile elongation and timings of start of the coleoptile splitting and the induction of Senescence-Associated Genes（SAGs）, both of which are senescence associated markers, were delayed in *d3* grown under darkness, but not under light conditions. These results indicate that the phenotype of elongated coleoptile in *d3* is not due to enhancement of the coleoptile elongation, but is due to delay of the coleoptile senescence under darkness.

The phenotypes of the mesocotyl and coleoptile elongation under darkness were similar to the phenotypes observed in rice JA-deficient mutant *cpm1*. Thus, I examined whether JA is involved in the coleoptile and mesocotyl elongation in *d3* under darkness. Treatment of JA（500 nM MeJA）under darkness reduced the lengths of mesocotyl and coleoptile of *d3*, and their lengths were almost the same between *d3* and Shiokari, suggesting that the JA treatment can suppress the enhanced elongation of coleoptile and mesocotyl in *d3*. The LC-MS analyses showed that the JA amounts in the coleoptiles were comparable between *d3* and Shiokari. These results suggested that the phenotype of the enhanced mesocotyl and coleoptile elongation was not due to the difference of JA contents between *d3* and Shiokari, but rather due to reduced sensitivity of coleoptile and mesocotyl to JA in *d3* comparing to Shiokari. Thus, higher concentration of JA may be required for reduction of the lengths of coleoptile and mesocotyl in the *d3* mutant.

The LC-MS analyses showed that the content of ABA in the *d3* coleoptile was nearly two fold higher than that in the Shiokari coleoptile. It was likely to be due to the activation of the ABA biosynthesis and the repression of the ABA catabolism in *d3* mutant coleoptiles compared to that in Shiokari. On the other hand, the GA content in the *d3* coleoptile was about half of the GA content in the Shiokari coleoptile.

I also investigated sensitivity of the *d3* coleoptile to red light of far-red light by checking the rate of inhibition of coleoptile growth. The coleoptile of the *d3* mutant initially had only a slight responsiveness to red light comparing to the case of Shiokari, and then the *d3* coleoptile gained greater responsiveness during elongation of coleoptile.

In conclusion, I found that the *d3* mutant increased leaf longevity during senescence or cell death induced by darkness, JA and H_2O_2. The *d3* coleoptile and mesocotyl were highly elongated and the senescence of coleoptile in the *d3* mutant was also delayed under darkness, but not under light conditions. The *d3* coleoptile initially had only a slighter responsiveness to red light compared to wild-type, and gained greater responsiveness during elongation of coleoptile. This study suggested that the deficiency of D3 protein affected to seedling development and senescence of leaves and coleoptile as well as tillering and stature of plant in rice. The target protein of rice D3 is not known. Thus, future characterization of D3-target protein appears to be necessary to understand the D3-dependent control of senescence and seedling development and to understand the relationship among tillering and stature of plant, senescence and seedling development in rice.