論文の内容の要旨

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論文題目 **Cloning and characterization of genes involved in Fe-homeostasis in graminaceous plants**

論文題目　**イネ科植物の鉄-ホメオスタシスに関わる遺伝子の単離と解析**

Iron (Fe) is an essential element required for various cellular events in plants, including respiration, chlorophyll biosynthesis, and photosynthetic electron transport. Fe is also a component of the Fe-S cluster, which is present in numerous enzymes. Thus the acquisition of Fe from soil and its homeostasis is essential for normal plant growth. To understand the mechanisms of Fe acquisition and homeostasis, different genes involved in Fe-acquisition/homeostasis or Fe-deficiency induced stress tolerance were cloned from graminaceous plants. These include deoxymugineic acid synthase (DMAS) from rice (*OsDMAS1*), barley (*HvDMAS1*), wheat (*TaDMAS1*) and maize (*ZmDMAS1*) plants, glutathione reductase (GR) from barley (*HvGR1* and *HvGR2*) and glutathione transporter like gene (*OsGTL1*). The expression patterns of these genes and their possible roles in Fe-acquisition as well as homeostasis were revealed.

1). **Cloning and characterization of DMAS genes form graminaceous plants**

Graminaceous plants have evolved a unique mechanism to acquire Fe and secrete a family of small molecules, called mugineic acid family phytosiderophores (MAs) in response to Fe-deficiency. MAs are synthesized form L-methionine. Three molecules of S-adenosyl methionine are combined together to form one molecule of nicotianamine (NA). The amino group of NA is transferred by NA-amino transferase to form Keto form, which is subsequently reduced to deoxymugineic acid (DMA) by deoxymugineic acid synthase (DMAS). DMA is the first member of MAs and MAs share the same pathway from L methionine to DMA and the

subsequent steps may differ depending upon the plant species and even cultivars. Previously all the genes cloning of DMAS, with the exception of DMAS, involved in MAs biosynthetic pathway have been cloned from rice and barley. *DMAS* was first isolated from rice (*OsDMAS1*) as a member of aldo-keto reductase super family (AKR) upregulated under Fe-deficiency and then its orthologs from barley (*HvDMAS1*), wheat (*TaDMAS1*), and maize (*ZmDMAS1*) were also cloned. Their nucleotide sequences indicate that *OsDMAS1* encodes a predicted polypeptide of 318 amino acids, whereas the other three orthologs all encode predicted polypeptides of 314 amino acids and are highly homologous (82-97.5%) to each other. The *DMAS* genes belong to AKR and have homology to *Papaver somniferum* codeinone reductases (AKR4B2-3), and *Medicago sativa* (AKR4A2) and *Glycine max* (AKR4A1) chalcone polyketide reductases (**Fig-1**). Although strictly speaking it does not fall in to existing subfamilies of AKR4, however, after introduction of conservative substitutions, the identity with existing members of AKR4B reached up to 65% and ZmDMAS1, OsDMAS1, HvDMAS1 and TaDMAS1 were assigned the numbers from AKR4B5 to AKR4B8 respectively. DMAS proteins were expressed in *E. coli.* as maltose binding fusion proteins and all the recombinant proteins showed DMA synthesis activity *in vitro*. Their enzymatic activities were highest at pH 8 to 9 (**Fig-2**), consistent with the hypothesis that DMA is synthesized in subcellular vesicles. Northern blot analysis revealed that the expression of each of the above *DMAS* genes is upregulated under Fe-deficient conditions in root tissue, and that of *OsDMAS1* and *TaDMAS1* are upregulated in shoot tissue. Western blot analysis confirmed that expression

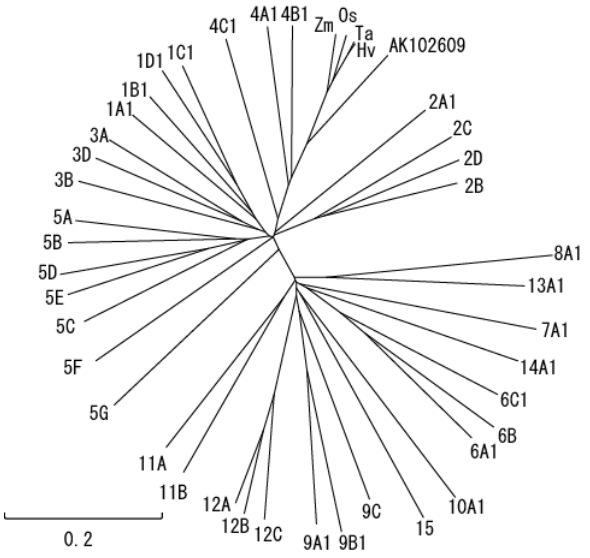

Fig. 1. Unrooted phylogenetic tree of the aldo-keto reductase superfamily (AKR)

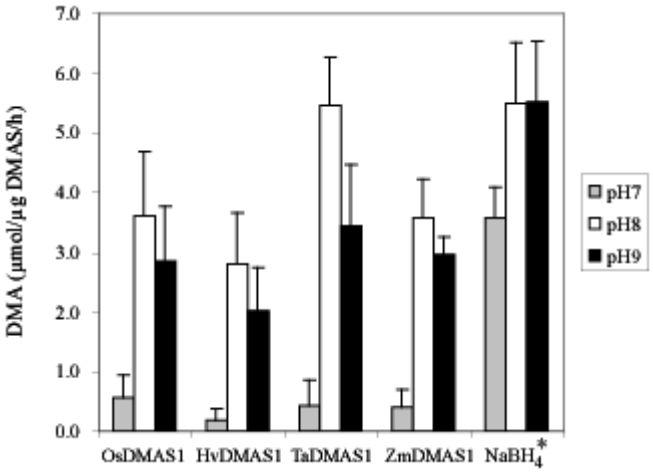

Fig-2. Effect of pH on enzyme activity of recombinant DMAS proteins

of DMAS is upregulated under Fe-deficiency and it seems that DMAS is not regulated posttranscriptionally. Moreover, it seems that low expression of DMAS is a rate limiting factor responsible for the low production of MAs in rice. *OsDMAS1* promoter-*GUS* analysis in Fe-sufficient roots showed that its expression is restricted to cells participating in long-distance transport, and that it is highly upregulated in entire root under Fe-deficient conditions. In shoot tissue, *OsDMAS1* promoter drove expression in vascular bundles specifically under Fe-deficient conditions. With the cloning of graminaceous DMAS, all the genes of MA biosynthetic pathway have been cloned form barley and rice. The cloning of DMAS is an important step in understanding the Fe acquisition and will help to develop transgenic rice highly resistant to Fe-deficiency in alkaline soils.

2). Cloning of glutathione reductase from barely

Glutathione reductase (GR) plays an important role in the response to biotic and abiotic stresses in plants like salt stress, high temperature, low temperature and pathogen attack. Although GR is also involved in response to Fe-toxicity, little is know about expression patterns of GR under Fe-deficient conditions. The expression patterns and enzyme activities of GR in graminaceous plants under Fe-sufficient and Fe-deficient conditions were examined by isolating cDNA clones for chloroplastic GR (*HvGR1*) and cytosolic GR (*HvGR2*) from barley. It was found that the sequences of *GR1* and *GR2* were highly conserved in graminaceous plants. Based on their nucleotide sequences, *HvGR1* and *HvGR2* were predicted to encode polypeptides of 550 and 497 amino acids, respectively. Both proteins showed *in vitro* GR activity, and the specific activity for HvGR1 was threefold that of HvGR2. Northern blot analyses were performed to examine the expression patterns of GR1 and GR2 in rice (*Oryza sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and maize (*Zea mays*). *HvGR1*, *HvGR2*, and *TaGR2* were upregulated in response to Fe-deficiency. Moreover, *HvGR1* and *TaGR1* were mainly expressed in shoot tissues, whereas *HvGR2* and *TaGR2* were primarily observed in root tissues. It was observed that the expression of *HvGR2* follows a diurnal rhythm in Fe-sufficient and Fe-deficient plants. The GR activity increased in roots of barley, wheat, and maize and shoot tissues of rice, barley, and maize in response to Fe-deficiency. Furthermore, it appeared that GR was not posttranscriptionally regulated, at least in rice, wheat, and barley. These results suggest that GR may play a role in the Fe-deficiency induced stress response in graminaceous plants.

3). Cloning and Characterization of Os*GTL1*

Glutathione (GSH) is involved in many aspects of plant growth and development including redox control, storage and transport of reduced sulfur, and response to biotic and

abiotic stresses. The transport and compartmentalization of GSH is essential to perform all these functions. A GSH transporter (GT) like genes was cloned from rice (*OsGTL1*). *OsGTL1* is a putative member of oligopeptide transporters (OPT) family, and was identified through microarray analysis as its expression was highly upregulated in response to Fe-deficiency in root and shoot tissue. *OsGTL1* was predicted to encode a polypeptide of 757 amino acids containing 12 putative transmembrane domains. It contains the NPG domain (NPGPFxxKEH) and KP domain (KLGHYMKIPPR) earlier identified in AtOPTs. Seven homologs of *OsGTL1* were identified in rice including previously characterized *OsGT1*. *OsGTL1* showed high homology i.e. 82% homology to *BjGT1* and 80% homology to *AtOPT3*. Northern blot analysis confirmed that the expression of *OsGTL1* is induced in response to Fe deficiency. OsGTL1:green fluorescent protein (GFP) was localized to the plasma membrane of onion epidermal cell. Electrophysiological measurements using *Xenopus leavis* oocytes showed that OsGTL1 is a functional GSH transporter. GUS expression driven by *OsGTL1* promoter was not observed in Fe-sufficient roots. In contrast, in Fe-deficient roots, high level of *OsGTL1* promoter derived GUS expression was observed near root tips and the expression diluted as the distance from root tip increased. In shoot tissue, *OsGTL1* promoter's expression was observed in whole leaf under Fe-deficient and specifically in vascular bundle under Fe-sufficient conditions. These results suggested that *OsGTL1* is a novel glutathione transporter up-regulated under Fe-deficient conditions and may play a critical role in Fe-deficiency induced stress in rice.

References

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