Title

Analysis of the spatial expression patterns of rice iron deficiency-responsive element-binding factors IDEF1 and IDEF2

Authors

Ogo, Yuko
Aung, May Sann
Kobayashi, Takanori
et al.

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Introduction

One-third of the fields on earth contain calcareous soil. Plants grown in calcareous soils that are low in iron (Fe) availability demonstrate decreased growth and yield. Under conditions of low Fe availability, rice plants induce transcriptional responses that promote the uptake of Fe from the soil as ferric Fe–mugineic acid phytosiderophore chelates and ferrous Fe ions. Thus, an understanding of the mechanisms by which plants such as rice respond to Fe deficiency is required to maintain plant yields and prevent food shortages.

In our previous studies, Kobayashi et al. (2003) identified two Fe-deficiency-responsive *cis*-acting elements (IDE1 and IDE2), which confer Fe deficiency-induced expression in rice roots and leaves (Kobayashi et al. 2004). We also found two rice transcription factors, IDE-binding factors 1 and 2 (IDEF1 and IDEF2, respectively), that bind to IDE1 and IDE2, respectively (Kobayashi et al. 2007; Ogo et al. 2008). IDEF1 and IDEF2 belong to uncharacterized branches of the plant-specific transcription factor families ABI3/VP1 and NAC, and they exhibit novel sequence recognition properties. *IDEF1* and *IDEF2* transcripts are constitutively expressed in both roots and leaves. Transgenic rice plants that express *IDEF1* under the control of the *IDS2* promoter were found to be tolerant to early Fe deficiency in hydroponic culture and calcareous soil. IDEF1 regulates the ferrous ion transporter gene *OsIRT1,* the Fe-deficiency-induced transcription-factor gene *OsIRO2*, and other genes related to Fe deficiency. IDEF2 regulates the metal nicotianamine transporter gene *OsYSL2* as well as other Fe-deficiency-related genes. Nevertheless, the specific mechanisms and tangential pathways affected by these key transcription factors have not been elucidated fully. Therefore, delineation of the expression patterns and characteristics of IDEF1 and IDEF2 could help elucidate the response of rice plants to Fe deficiency. To understand the mechanisms by which plants respond to Fe deficiency, we examined the expression patterns of *IDEF1* and *IDEF2* by promoter–GUS analysis under Fe-sufficient and Fe-deficient conditions. This information could be critical for the creation of rice varieties that grow in problem soils.

Materials and Methods

We analyzed the spatial expression patterns of *IDEF1* and *IDEF2* during the germination, vegetative, and seed-maturation stages by histochemical localization of GUS staining as described by Inoue et al. (2003) and Nozoye et al. (2007). Two kb of the 5' region upstream of the translation start site was used as the promoter sequence for *IDEF1*, whereas 2 kb of the 5' region upstream of the transcription start site was used as the promoter sequence for *IDEF2.* Rice (cultivar Tsukinohikari) was transformed with the *IDEF1* promoter–*GUS* or the *IDEF2* promoter–*GUS* by an *Agrobacterium*-mediated method, and T_1 or T_2 seeds were obtained for use in the analysis.

To induce Fe deficiency, 28-day-old plants were cultivated hydroponically without Fe–EDTA 1-12 days before harvest. For analysis during the flowering and maturing periods, *IDEF1* and *IDEF2* seeds were cultured in Fe-sufficient artificial soil with fertilizer, and developing seeds were progressively sampled for GUS expression analysis.

Results and Discussion

Expression pattern of IDEF1 and IDEF2 during the germination stage

IDEF1 and *IDEF2* expression was observed in both the endosperm and embryo during the early seed germination period. *IDEF2* expression was induced in the leaf primordium during germination.

IDEF1 and IDEF2 regulate genes related to Fe deficiency as well as other unknown genes. The expression patterns of some Fe-deficiency-induced genes have been investigated during the early germination period (Nozoye et al. 2007). The expression patterns of *IDEF1, IDEF2,* and *OsNAS1* were similar; all were expressed in the embryo and endosperm. Conversely, other Fe-deficiency-induced genes such as *OsNAS2*, *OsNAS3*, *OsNAAT1*, *OsDMAS1*, *OsYSL2,* and *OsIRT1* were not expressed in endosperm tissues. It is speculated that genes involved in phytosiderophore biosynthesis and Fe transport are differentially regulated in the germination and vegetative stages under low Fe conditions.

Expression pattern of IDEF1 and IDEF2 during the vegetative stage

IDEF1 or *IDEF2* promoter–*GUS* plants were grown in hydroponic culture under Fe-deficient or Fe-sufficient conditions. The expression patterns of *IDEF1* and *IDEF2* were found to be similar despite Fe availability. In leaf blades of *IDEF1* lines, strong expression was observed in mesophyll cells and in small vascular bundles. Interestingly, the main vascular bundle was not stained in either Fe-sufficient or Fe-deficient leaf samples. It is assumed that the principle function of the main vascular bundle is to transport water and nutrients and that Fe is needed in mesophyll cells and small vascular bundles for photosynthesis. In contrast to *IDEF1*, *IDEF2* was highly expressed in vascular bundles but not in mesophyll cells.

In the inner layers of the stem/leaf sheath of *IDEF1* lines, mesophyll and small vascular cells demonstrated dense staining, indicating high *IDEF1* expression. In root sections, *IDEF1* and *IDEF2* lines showed strong GUS staining in the secondary roots, which emerge under conditions of Fe deficiency. This finding suggests that IDEF1 and IDEF2 induce Fe-deficiency-responsive genes such as *OsIRT1* and *OsIRO2* in secondary roots and that this may play an important role in the uptake and utilization of Fe in low Fe conditions. GUS staining was also found inside the vascular bundles of root sections in *IDEF1* and *IDEF2* lines.

Expression pattern of IDEF1 and IDEF2 during the flowering and maturation stages

IDEF1 and *IDEF2* lines were cultured in soil to investigate spatial expression patterns during the flowering and maturation periods. Prior to anthesis, *IDEF1* line pollen showed high expression. After fertilization, the ovary was found to be heavily stained. Expression was also observed in the vascular bundles of the husk during the flowering and early seed development stages. There was strong staining of the embryo and the aleurone layer in the late progress maturation stages (e.g., 10, 15, and 30 days after fertilization). In embryos, the scutellum and leaf primordium were densely stained.

Similar to *IDEF1*, *IDEF2* was expressed in most pollen in the flowering stage. *IDEF2* was also expressed in immature seeds just after flowering and in the dorsal vascular sections in the late maturation stage.

Conclusions

Promoter–GUS analysis revealed that *IDEF1* and *IDEF2* are constitutively expressed during the germination, vegetative, and reproductive periods. The spatial expression patterns of *IDEF1* and *IDEF2* partially overlapped with their target genes. Further investigations are needed to clarify the precise mechanisms that regulate the responses of plants to Fe deficiency.

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