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Dual regulation of iron deficiency response mediated by the transcription factor IDEF1

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Higher plants respond to fluctuating Fe availability by regulating the expression of genes involved in Fe homeostasis. Transcriptional responses to Fe deficiency in plants are mediated via various interactions between cis-acting elements and trans-acting factors. The transcription factor IDEF1 regulates the response to Fe deficiency in *Oryza sativa* (rice) by recognizing the CATGC sequence within the Fe deficiency-responsive cis-acting element IDE1. We recently presented evidence that *IDEF1* mediates two-phase responses to Fe deficiency. During the early stages of Fe deficiency, the majority of known Fe uptake/utilization-related genes are positively regulated by IDEF1. In subsequent stages, IDEF1-mediated regulation of these Fe uptake/utilization-related genes are less obvious. In turn, expression of several Fe deficiency-induced genes encoding late embryogenesis abundant proteins is increasingly regulated by IDEF1 at the subsequent stages. We propose a dual function of IDEF1 for Fe deficiency response, namely, (1) the coordinated transactivation of Fe utilization-related genes via CATGC-containing IDE1-like elements, especially at the early stage, and (2) the transactivation of seed maturation-related genes via RY elements, especially during the subsequent stages of Fe deficiency. *IDEF1* appears to have evolved to mediate an interface between Fe deficiency-inducible and seed maturation-related gene expression.

Iron (Fe) is an essential mineral nutrient for all plants. To acquire enough Fe, while circumventing toxicity of excess Fe, plants tightly control the level of Fe uptake, utilization and storage in response to Fe availability in the environment. In spite of its high abundance in the Earth's crust, Fe is sparingly soluble in aerobic conditions, especially in calcareous and high pH soils.[1] Under conditions of low Fe availability, higher plants adopt two major strategies for Fe acquisition from the rhizosphere: Strategy I, utilized by non-graminaceous plants, and Strategy II by graminaceous plants.[2] In both strategies, expression of the genes participating in Fe acquisition is strongly induced in response to Fe deficiency. Molecular components regulating these Fe deficiency responses are now being elucidated. The Strategy I response is mediated by Fe deficiency-inducible basic helix-loop-helix (bHLH) transcription factors (TFs), among which tomato FER and its *Arabidopsis thaliana* ortholog FIT (formerly FIT1/FRU/AtbHLH29) are the most characterized.[3-5] Interactions of these bHLH TFs are thought to play key roles in regulation.[6] However, neither the functional cis sequences nor their upstream regulation pathways have been elucidated for these non-graminaceous TFs.

Our recent studies focused on clarification of cis element/trans factor interactions regulating Fe deficiency responses of graminaceous genes. Promoter analysis of the barley Fe deficiency-inducible *IDS2* gene led to identification of the Fe deficiency-responsive cis-acting elements IDE1 and IDE2,[7] which are functional both in rice (Graminaceae) and tobacco (non-Graminaceae).[7,8] We further identified two rice IDE-binding TFs, IDEF1

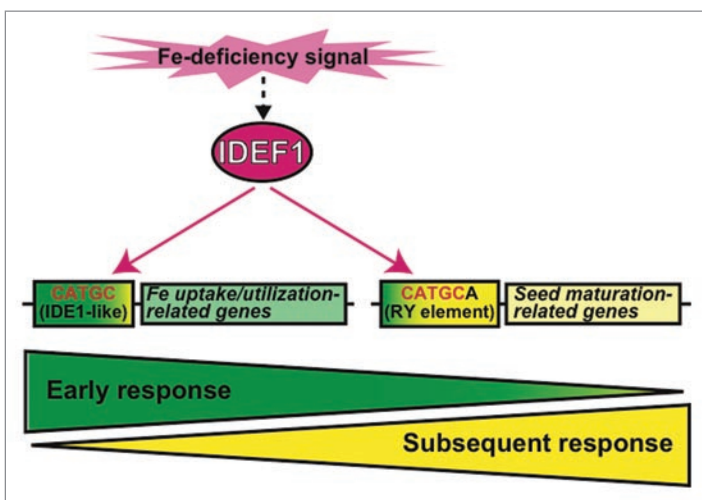

Figure 1. A simplified model of dual regulation of Fe deficiency response mediated by IDEF1.

(ABI3/VP1 family) and IDEF2 (NAC family), which specifically bind to IDE1 and IDE2, respectively.[9,10] Both *IDEF1* and *IDEF2* are constitutively expressed irrespective of Fe status, suggesting their roles in sensing Fe deficiency signals directly or indirectly. Characterization of transgenic rice plants with altered *IDEF1* or *IDEF2* expression levels revealed physiological functions of these TFs in Fe homeostasis during Fe deficiency.[9-11] RNAi-mediated *IDEF2* knockdown lines exhibit aberrant Fe distribution between roots and shoots and are defective in inducing a subset of Fe deficiency-induced genes.[10]

Transgenic rice lines with an introduced *IDEF1* under the control of the Fe deficiency-inducible *IDS2* promoter exhibit a slower decrease in leaf chlorophyll under low Fe availability and enhanced expression of an Fe deficiency-induced bHLH TF gene *OsIRO2*.[9] OsIRO2 binds to the CACGTGG sequence[12] and regulates the majority of the genes involved in Strategy II Fe acquisition.[13] These findings indicate the presence of a gene regulation cascade involving IDEF1 and OsIRO2.[9,13]

Through time-course expression analysis of transformants with induced or repressed *IDEF1* expression, we previously revealed that IDEF1-mediated gene regulation consists of two phases during the progression of Fe deficiency.[11] On day 1 of Fe deficiency treatment in hydroponic culture, IDEF1 mediates transactivation of the majority of the Fe deficiency-induced genes currently known to be involved in Fe uptake and/or utilization. On day 2, and thereafter, IDEF1-mediated transactivation of these Fe uptake/utilization-related genes becomes weaker and restricted to only some of the members. In silico analysis of rice promoters revealed that *IDEF1* target genes, as estimated by microarray analyses, exhibit a significantly higher presence of IDEF1-binding core sites (CATGC) and IDE1-like sequences in the proximal promoter regions. The highest occurrence of these sequences was found on day 1 of Fe deficiency, confirming the predominance of an early response that is mediated, at least in part, through direct binding of IDEF1 to CATGC sequences (**Fig. 1**, left).

We further investigated the occurrences of the RY element (CATGCA) among the *IDEF1* target genes, because the RY element is thought to be the minimal recognition sequence of all the ABI/VP1 family TFs analyzed,[14-16] except for the IDEF1 subfamily.[9] Interestingly, *IDEF1* target gene promoters possess not only CATGC but also RY elements at significantly high levels, even though IDEF1 does not require the sixth base of the RY element (CATGCA) for efficient binding in vitro.[9] In general, RY elements confer seed-specific expression of various genes, including those encoding late embryogenesis abundant (LEA) proteins.[16] Our expression analyses revealed that a subset of *LEA* genes including *Osem* is induced even in vegetative organs (leaves and roots) in response to Fe deficiency in an IDEF1-dependent manner, especially at the subsequent stages (**Fig. 1**, right). Thus, IDEF1 is thought to possess a dual function for Fe deficiency response: the coordinated transactivation of Fe uptake/utilization-related genes via CATGC-containing IDE1-like elements, and the transactivation of seed maturation-related genes via RY elements. The former is predominant at the early stage, while the latter becomes dominant at subsequent stages of Fe deficiency.

Because IDEF1 is expressed during both vegetative and reproductive stages,[17] this TF might positively regulate seed maturation-related genes in various organs. In addition, the unique DNA-binding property of IDEF1 to efficiently bind to CATGC and IDE1-like elements lead to this TF being capable of regulating the Fe deficiency response. Thus, IDEF1 appears to have evolved to mediate an interface between Fe deficiency-inducible and seed maturation-related gene expression. Curiously, gene homologs of the IDEF1 subfamily have been found only in graminaceous plants, even though IDE1 is also functional in non-graminaceous tobacco plants.[7,9]

The essentiality of IDEF1 for Fe deficiency response is also confirmed by the phenotype of *IDEF1* knockdown lines, which are hypersensitive to Fe deficiency, as observed by an earlier decrease in leaf chlorophyll.[11] Clarification of factors interacting with IDEF1 and/or IDEF2 might provide a significant step toward our understanding of the precise mechanisms of Fe deficiency response in plants, including the nature of the Fe deficiency signal. In addition, the IDEF1-mediated transactivation of seed maturation-related genes suggests possible roles for these genes under low Fe availability, thus providing a basis for exploring new functions for LEA proteins.

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