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Tissue-specific transcriptional profiling of iron-deficient and cadmium-stressed rice using laser capture microdissection

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Abbreviations: Fe, iron; MA, mugineic acid; YSL, yellow stripe 1-like; DC, discrimination center; Cd, cadmium; LM, laser microdissection; OPT, oligopeptide transporter; MFS, major facilitator superfamily; NRAMP, natural resistance-associated macrophage protein; ZIP, zinc-regulated transporter, iron-regulated transporter-like proteins; IRT1, Iron-Regulated Transporter 1; HMA, Heavy Metal ATPase

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Several metals are essential nutrients for plants. However, they become toxic at high levels and deleteriously affect crop yield and quality. We recently reported the spatial gene expression profiles of iron (Fe)-deficient and cadmium (Cd)-stressed rice using laser microdissection and microarray analysis. The roots of Fe-deficient and Cd-stressed rice were separated into the vascular bundle (VB), cortex (Cor), and epidermis plus exodermis (EP). In addition, vascular bundles from new and old leaves at the lowest node, which are important for metal distribution, were analyzed separately (newDC and oldDC, respectively). Genes expressed in a tissue-specific manner in the VB, Cor, EP, newDC, and oldDC formed large clusters. The genes upregulated in all of the VB, Cor, and EP by Fe deficiency formed a substantial cluster that was smaller than the tissue-specific clusters. Significant numbers of genes expressed in newDC or oldDC were also expressed in VB in roots, suggesting that vascular bundles in the lowest nodes and roots have a partially common function. The expression patterns of transporter families involved in metal homeostasis were investigated, and members of each family were either expressed differentially in each tissue or showed different responses to Fe deficiency. One potassium transporter gene, *OsHAK22*, was upregulated by Fe deficiency in VB, Cor, and EP, suggesting that OsHAK22 is involved in potassium transport associated with mugineic acids secretion.

The availability of essential metals such as iron (Fe), zinc (Zn), manganese (Mn), and copper severely affects crop yield and quality. Under conditions of low Fe availability, graminaceous plants utilize Fe(III) chelaters known as mugineic acid family phytosiderophores (MAs) to absorb Fe.[1] Biosynthesized MAs are secreted into rhizospheres through TOM1,[2] where they chelate Fe(III). The resulting Fe(III)–MAs complex is taken up via yellow stripe 1-like (YSL) family transporters.[3] The expression of many genes involved in MAs biosynthesis and Fe transport are upregulated coordinately in response to Fe deficiency in rice. Kobayashi et al.[4,5] and Ogo et al.[6,7] demonstrated that the transcription factors IDEF1, IDEF2, and OsIRO2 regulate crucial steps of gene regulation in response to Fe deficiency.

In contrast, cadmium (Cd) is toxic to living organisms. Cd-polluted soil, which is found across a wide global area and causes Cd accumulation in crops, is a growing threat to agriculture and human health. Cd-induced toxicity in plants disturbs the balance of essential metals in metalloenzymes.[8,9] Cd is thought to be absorbed and translocated by the transporters of essential metals, including Fe, Zn, and Mn, which have chemical properties similar to those of Cd. Recently, several metal transporters such as OsNRAMP5 were shown to play a key role in Cd uptake from the soil into rice.[10-12]

The molecular mechanisms underlying the absorption of Fe and Cd from the

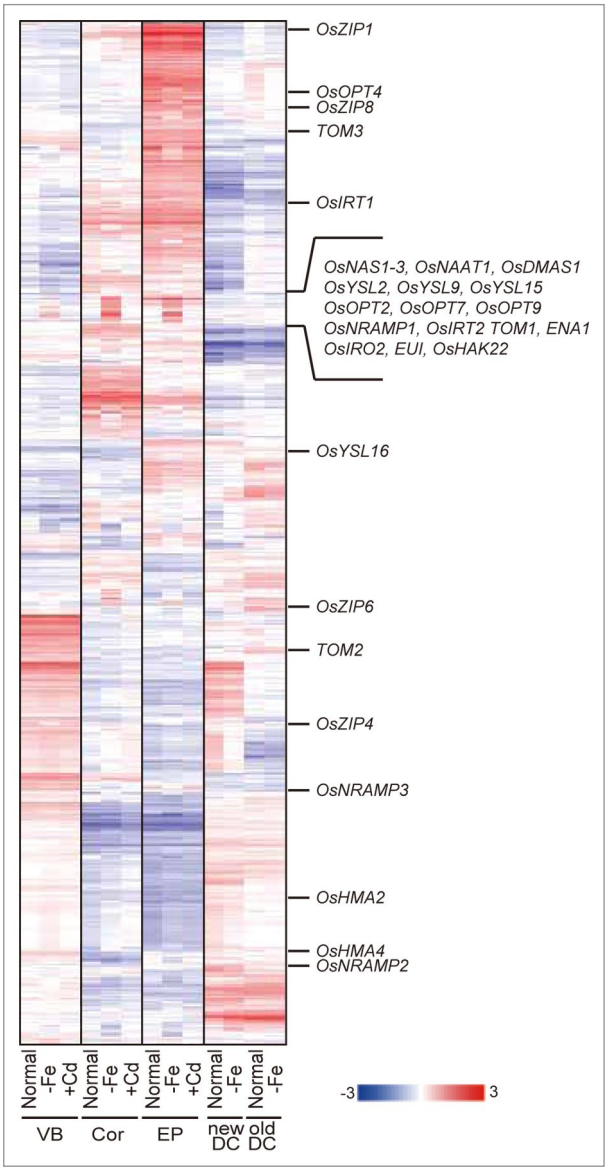


Figure 1. Expression profiles of the genes involved in metal homeostasis. Clustering analysis of genes whose expression changed in at least one tissue or under one condition. The gene-normalized signal intensities are shown in heat maps using a $\log_{10}$ scale. Hierarchical clustering was performed as described previously.[13] OsNAS1–3, nicotianamine synthase; OsNAAT1, nicotianamine aminotransferase; OsDMAS1, deoxymugineic acid synthase; OsYSL2, Fe(II)–nicotianamine synthase; OsYSL15, 16, Fe(III)–DMA transporter; OsYSL9, YSL transporter with unknown substrates; OsIRT1, 2, Fe(II) transporter; TOM1, DMA efflux transporter; TOM2, 3, homologous genes of TOM1; ENA1, nicotianamine efflux transporter; OsIRO2, Fe deficiency-inducible transcription factor; OsNRAMP1, Fe transporter with broad substrates; OsNRAMP2 and 3, NRAMP transporters with unknown substrates; OsZIP4 and 8, Zn transporters; OsZIP1 and 6, ZIP transporters with unknown substrates; OsHMA2, Zn/Cd transporter; OsHMA4, HMA transporter with unknown substrates; EUI, gibberellin-deactivating enzyme; OsHAK22, high-affinity potassium (K^+) transporter.

soil and their distribution in plants are being explored by analyzing individual genes. However, numerous genes related to metal homeostasis remain uncharacterized. Therefore, we investigated the tissue expression profiles and changes in expression during the response to Fe deficiency and Cd stress using a combination of laser microdissection (LM) and rice microarrays.[13] Genes encoding transporters involved in metal homeostasis, proteins associated with heavy metal detoxification, and phytohormone-related proteins were then investigated comprehensively. Rice roots grown under normal, Fe-deficient, or Cd-stressed conditions were separated by LM into 3 distinct tissue types: vascular bundles (central cylinder; VB), cortex (Cor), and exodermis plus epidermis (EP). In graminaceous plants, the lowest node of the shoots, known as the discrimination center (DC), is important for the distribution of metals and metabolites to leaves,[14-16] and usage of xylem and/or phloem in Fe transport differs between new and old leaves.[17] Therefore, vascular bundles in the DC from new and old leaves (newDC and oldDC, respectively) under normal and Fe-deficient conditions were isolated separately. RNA from each tissue was then extracted from 3 biological replicates, and 44 K rice microarrays were analyzed.

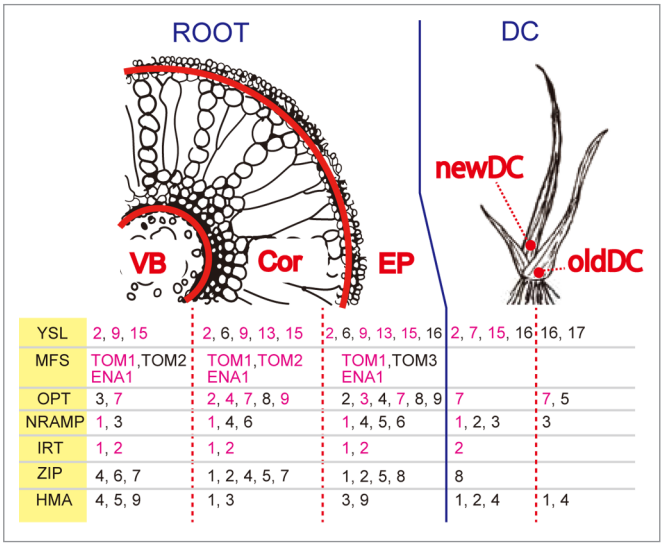
Clustering analysis was performed on the genes whose expression changed in at least one tissue or under one condition (**Fig. 1**). The large clusters formed by genes expressed in a tissue-specific manner in the VB, Cor, EP, newDC, or oldDC revealed a specific function of each tissue, such as long-distance transport, radial transport, absorption/secretion, and distribution to new or old leaves, respectively. Many genes expressed in the vascular bundles in DC (newDC and oldDC), particularly the newDC, were also expressed in the VB, although there were also numerous genes that were expressed differentially in the newDC/oldDC and VB (**Fig. 1**). These suggest that vascular bundles in DC and roots might have a common function, such as the long-distance transport of various kinds of molecules but also have distinct functions. The genes upregulated by Fe deficiency in all of VB, Cor, and EP formed a smaller cluster than the tissue-specific clusters, but it remained substantial. These genes included those of MAs biosynthetic enzymes, the transporters involved in Fe uptake, the Fe-deficiency inducible transcription factor *OsIRO2*, and the gibberellin-deactivating enzyme *EUI* (**Fig. 1**).

The expression patterns of the transporter families involved in metal homeostasis, YSL transporters, oligopeptide transporters (OPTs), major facilitator superfamily (MFS) antiporters, natural resistance-associated macrophage proteins (NRAMP), zinc-regulated transporter, iron-regulated transporter-like proteins (ZIPs), and heavy metal ATPase (HMA)

were investigated. Their expressed tissues and expression changes caused by Fe deficiency are shown in **Figure 2**. Members of each family were either expressed differently in each tissue, or they showed different responses to Fe deficiency. The transporters expressed in a tissue-specific manner in the VB, Cor, and EP might be involved specifically in long-distance transport (OsNRAMP3, OsZIP6, OsHMA4, and OsHMA5), radial transport, and metal absorption (OsYSL16, TOM3, OsNRAMP5, and OsZIP8), respectively. The transporters expressed in newDC and oldDC might be involved in distributing metals to new leaves (OsYSL2, 7, 15, OsNRAMP1, 2, OsIRT2, OsZIP8, and OsHMA2) and old leaves (OsYSL17, OsOPT5), respectively. The upregulation of certain transporters by Fe deficiency in various tissues (such as OsYSL2, OsIRT2, TOM1, and ENA1) suggests that they are involved both in Fe uptake and translocation.

Numerous genes were upregulated by Fe deficiency in various tissues; the effect of several of these genes on Fe deficiency has not yet been elucidated. Among these, the potassium (K) transporter *OsHAK22* was upregulated by Fe deficiency in the VB, Cor, and EP, whereas the other *OsHAK*s were not upregulated significantly by Fe deficiency in any tissue (**Fig. 3**). *OsHAK22* is also upregulated during the early stages of Fe deficiency.[17] MAs are thought to be secreted as monovalent anion, and equamoler potassium was secreted while MAs secretion.[19-21] Fe-deficient barley roots contain more K than Fe-sufficient barley roots, and K in barley roots is dramatically decreased after secretion of MAs,[22] suggesting that a large amount of K is transferred into and from root cells during Fe deficiency. However, the precise mechanism of K secretion was unclear. The expression pattern of *OsHAK22* was similar to that of the MAs efflux transporter *TOM1*, which is upregulated by Fe deficiency in the VB, Cor, and EP. OsHAK22 is thought to be involved in the K transport associated with MAs secretion and is expressed in a coordinated manner with TOM1.

In conclusion, this study provides useful information for understanding the molecular mechanisms involved in metal absorption, transport, and distribution, and makes a significant contribution to identifying the tissue-specific responses to metal stress.



	VB	Cor	EP	newDC	oldDC
YSL	2, 9, 15	2, 6, 9, 13, 15	2, 6, 9, 13, 15, 16	2, 7, 15, 16	16, 17
MFS	TOM1,TOM2 ENA1	TOM1,TOM2 ENA1	TOM1,TOM3 ENA1		
OPT	3, 7	2, 4, 7, 8, 9	2, 3, 4, 7, 8, 9	7	7, 5
NRAMP	1, 3	1, 4, 6	1, 4, 5, 6	1, 2, 3	3
IRT	1, 2	1, 2	1, 2	2	
ZIP	4, 6, 7	1, 2, 4, 5, 7	1, 2, 5, 8	8	
HMA	4, 5, 9	1, 3	3, 9	1, 2, 4	1, 4

Figure 2. Schematic representation of the tissue expression and expression changes by Fe deficiency of representative transporters involved in metal homeostasis. VB, vascular bundle; Cor, cortex; EP, exodermis plus epidermis; newDC, vascular bundles from new leaves in the DC; oldDC, vascular bundles from old leaves in the DC. The genes listed in the Table are expressed in the indicated tissues. Genes in pink and black letters are upregulated and not upregulated by Fe deficiency, respectively, in the indicated tissue.

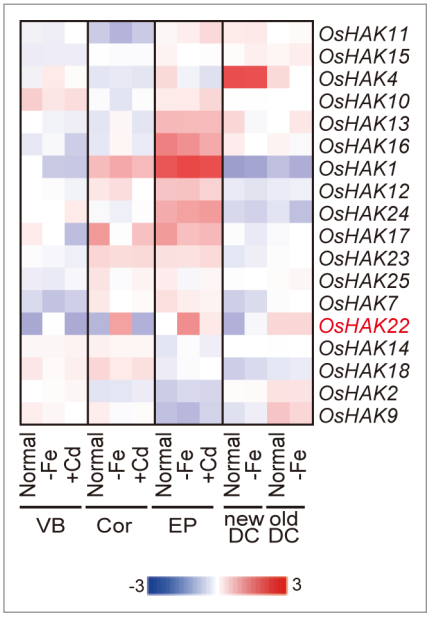


Figure 3. Expression profiles of the KT-HAK-KUP potassium transporter family in rice. The rice KT-HAK-KUP family genes (OsHAK1–25) were described by Amrutha et al.[18]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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