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The role of rice phenolics efflux transporter in solubilizing apoplasmic iron

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Iron (Fe) is an essential micronutrient for plants whose deficiency presents a major worldwide agricultural problem. Moreover, Fe is not easily available in neutral to alkaline soils, rendering plants deficient in Fe despite its abundance. Plants secrete phenolics, such as protocatechuic acid (PCA) and caffeic acid (CA), to take up and utilize apoplasmic precipitated Fe, but despite the rapid progress in understanding cellular and subcellular Fe transport, the molecular mechanisms of phenolics synthesis and secretion are not clear. Recently, we isolated and characterized a phenolics efflux transporter in rice by characterizing a mutant in which the amount of PCA and CA in the xylem sap was dramatically reduced, which we hence named *phenolics efflux zero 1* (*pez1*). PEZ1 is a plasma membrane protein that transports PCA when expressed in *Xenopus laevis* oocytes, and characterization of *PEZ1* knockdown and overexpressing plants revealed that it plays an essential role in solubilizing precipitated apoplasmic Fe. The identification of PEZ1 will increase our understanding of apoplasmic Fe solubilization as well as promote research on phenolics efflux mechanisms in different organisms.

Key words: iron, *Oryza sativa*, phenolics transport, protocatechuic acid, xylem sap

Abbreviations: IRT1, iron-regulated transporter; PCA, protocatechuic acid; PEZ1, phenolics efflux zero 1

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Although mineral soils contain over 6% iron (Fe),[1] it predominantly exists as Fe(III) chelates, and plants ultimately cannot absorb Fe under various physiological conditions such as high soil pH in alkaline soils.[2] Thus, plants growing in high-pH soils are not very efficient in developing and stabilizing chlorophyll, resulting in the yellowing of leaves, poor growth and reduced yield. Plants, however, have developed sophisticated mechanisms to take up the small amount of soluble Fe. Non-graminaceous plants release protons, secrete phenolics, reduce Fe(III), and finally, take up Fe^{2+}.[3-5] Once Fe is solubilized, Fe(III) is reduced to Fe^{2+} by a membrane-bound Fe(III) reductase oxidase.[6] Then Fe^{2+} is transported into the root by an iron-regulated transporter (IRT1). In contrast, graminaceous plants rely on an Fe(III) chelation system through the secretion of mugineic acid (MA) family phytosiderophores.[3,7,8] The MAs are secreted to the rhizosphere through TOM1 [9] and then they chelate Fe(III); the resulting Fe-MA complex is transported by the Yellow Stripe family transporters (OsYSL15 in the case of rice[10]). Rice plants also have the ability to take up Fe^{2+} through the OsIRT1 transporter.[11]

In plants, Fe uptake from the apoplasm is well documented at the molecular level, with the exception of phenolics synthesis and efflux. Phenolics, such as protocatechuic acid (PCA), are reported to chelate Fe(III) solubilization and reduce it to Fe^{2+} in vitro.[12] Moreover, removing the secreted phenolics in hydroponic culture solution triggers Fe deficiency responses in roots by inhibiting the solubilization and utilization of apoplasmic Fe.[13] In this manner, phenolics play a major role in Fe solubilization, besides which PCA and other phenolics play a diverse role in biological systems, such as acting as antioxidants and

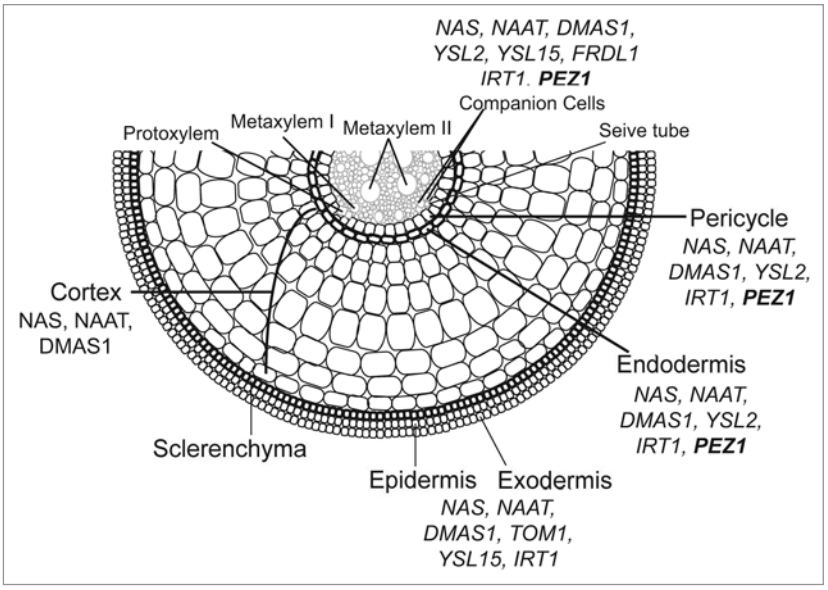

Figure 1. Tissue-specific expression of Fe homeostasis-related genes in rice root.

free radical scavengers, and in nitric oxide synthase.[14-17] Phenolics are also converted to lignin and suberin through the action of peroxidases.[2] The activity of peroxidases, as well as the formation of lignin, decreases under Fe deficient conditions.[2,18] As suberin plays an important role in controlling the movement of solutes,[19] the role of phenolics in controlling water and mineral transport cannot be overlooked. Thus, understanding the molecular mechanism of phenolics efflux transport is crucial for developing strategies to mitigate widespread Fe deficiency.

PEZ1 was isolated in an effort to characterize T-DNA mutants for genes regulated by cadmium (Cd). PEZ1 belongs to the multidrug and toxic compound extrusion transporter family whose members transport small organic compounds.[20] The substrates of PEZ1 were identified by analyzing liquid chromatography/mass spectrometry data profiles of the xylem sap of *pez1-1* and *pez1-2* mutants. The data indicated that PEZ1 transports PCA and caffeic acid (CA). Furthermore, PEZ1 transported radiolabeled PCA when expressed in *Xenopus laevis* oocytes. PEZ1 localizes to the plasma membrane in rice root cells, as well as in rice root hairs and onion epidermal cells. The *pez1-2* mutant accumulated more Fe in the roots, but not in the leaves, compared to wild-type (WT) plants; the differences were greater in the presence of Cd, while no difference was observed in the accumulation of other metals. No significant difference was observed in zinc, manganese (Mn), and copper concentration between WT and *pez1-2*, in both the roots and shoots, with or without Cd. Fe concentration in the xylem sap was lower than in the WT, while no difference was observed for xylem Cd and Mn. Significant differences in the localization of insoluble Fe were observed when leaf samples were stained with Perl's solution to examine the localization of Fe. These results suggested a clear role of PEZ1 in solubilizing precipitated apoplasmic Fe.[21]

Secretion of excess PCA strongly solubilizes Fe precipitated in the stele, leading to symptoms of Fe excess. The analysis of *PEZ1* overexpression lines confirmed this hypothesis. *PEZ1* overexpression lines accumulated higher amounts of Fe in roots and leaves owing to the high solubilization of precipitated apoplasmic stele Fe, and as a result, the growth of these lines was severely restricted. In contrast, *PEZ1* overexpression lines grew better than the WT in calcareous soil, showing that in these lines, PCA-solubilized Fe is available under Fe-limiting conditions.

The expression of PEZ1 is regulated by Cd, and both of the PEZ1 knockdown mutants accumulated higher Cd amounts in leaves and seeds when grown in soil, without compromising morphological or physiological characteristics, like the SPAD value, leaf dry weight, yield, and the concentration of other metals in seeds. Why *pez1* accumulates Cd is not clear. PCA has a lower affinity for Cd compared to glutathione, and PEZ1 does not transport Cd.[21] Cd is partly transported through the Fe uptake system in plants.[22-26] Thus, in *pez1*, Cd accumulation seems to be triggered by the upregulation of OsIRT1. OsIRT1 localization in the phloem, its substrate specificity, and increased expression in *pez1* mutants suggests that Fe and Cd uptake and translocation in *pez1* mutants could be enhanced through OsIRT1,[11] and that an increased Cd accumulation in *pez1* mutants may be due to the increase in OsIRT activity in a decreased Fe environment in which Cd will have reduced competition. PEZ1 localizes to the stele in root cells. The localization of different genes involved in Fe transport is summarized in **Figure 1**.

In short, phenolics secretion affects Fe acquisition in rice. Reduced secretion of PCA in the *pez1-2* mutant impairs the solubilization of precipitated apoplasmic Fe in the stele, and thus, the low availability of Fe leads to the induction of *OsIRT1*. As *PEZ1* and *OsIRT1* co-localize in the stele, the PCA secretion may complement Fe^{2+} uptake by OsIRT1 and seems to be an integral part of the Fe^{2+} uptake system in rice (**Fig. 2**). In contrast, the increase in phenolics secretion in PEZ1-overexpressing plants increases the solubilization of apoplasmic Fe, and plants showed an increased tolerance to Fe deficiency in alkaline soils. The identification of PEZ1 is an important step that helps in better understanding the solubilization of apoplasmic Fe and will generate research on phenolics efflux mechanisms in other plants.

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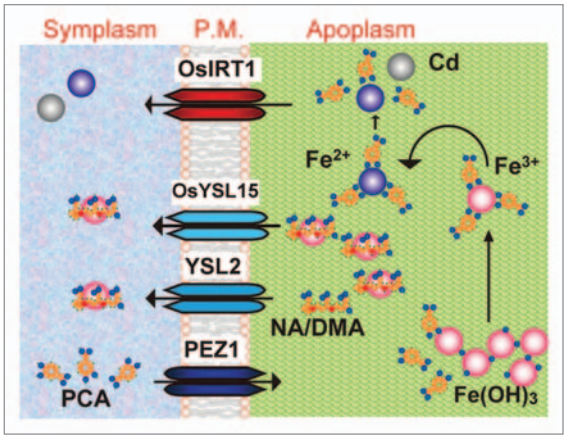

Figure 2. Model of Fe and Cd uptake mechanisms in rice xylem. P.M., plasma membrane.

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