Construction of cDNA library from iron-deficiency induced maize roots and screening and identification of iron-stress gene \textit{Fdr3}

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Abstract To isolate Fe-deficient related (\textit{Fdr}) genes, an expression cDNA library of \(4.5 \times 10^6\) pfu/\(\mu\)g has been constructed from maize roots in iron-stress. 6 clones have been screened from the cDNA library by differential hybridization screening. It is proved that an \textit{Fdr3} cDNA clone expressed stronger under iron-deficient condition than under iron-sufficient one by Northern blot and Western blot.

Keywords: maize, iron-deficient cDNA library, differential hybridization screening, iron-deficient related (\textit{fdr}) clone.

Iron (Fe) is one of the essential microelements for plant growth. However, available Fe element is often limited in calcific soils in China and other countries over the world. Two different strategies of root response to Fe deficiency have been identified in various plants\textsuperscript{[1]}. Strategy I occurs in all plants except grasses, in which plasma membrane-bound reductase activity is induced with enhanced net excretion of protons. In Strategy II\textsuperscript{[2]}, grasses respond to Fe-deficiency stress by enhancing the release of phytosiderophores (PS) which form chelate with Fe\textsuperscript{3+}. Uptake of Fe\textsuperscript{3+}-PS is mediated by a specific transporter in the plasma membrane of root cells of grasses. According to the amount of PS from high to low, the important crops are ordered by barley > rye > wheat > oat > maize > Chinese sorghum>> rice\textsuperscript{[3,4]}.

Maize was classified as Strategy II due to PS (2'-deoxymugineic acid DMA)\textsuperscript{[5]} which was secreted from roots in Fe-deficient. Von Wiren et al.\textsuperscript{[6]} confirmed that iron inefficiency in the maize mutant \textit{ysl} (Zea mays L. cv Yellow-Stripe) is caused by a defect in the uptake system for Fe(III)-PS. To characterize this defect further, Von Wiren designed an uptake experiment with double-labeled \(^{59}\)Fe-[\(^{14}\)C]-DMA by the Fe-efficient maize cultivator Alice and the Fe-inefficient mutant \textit{ysl}. The
results suggested that Fe-DMA was taken up by the roots as the intact chelate in both cultivators; moreover, they indicated the existence of a high- and a low-affinity uptake systems mediating Fe-PS transport across the root plasma membrane in maize. Apparently the mutation responsible for Fe-inefficiency in ysl affected the high-affinity uptake and led to the decrease in activity and/or number of Fe-PS transporters\[7\]. However, Baguaes et al.\[8\] proved that NADH-Fe (III)-EDTA reductase was induced under Fe-deficiency and led maize roots to uptake more iron. Fe (III) reductase activity on roots surface was enhanced and pH value decreased in maize (WP9) rhizosphere but Fe-PS has not been tested in this Fe-efficient cultivator. During intercropping between maize and peanut in calcific soil, the peanut did not show chlorosis as usual and the fact that the products of peanut had increased, indicated that the secreted material under Fe-deficiency in maize could be available for the iron uptake in dicotyledonous plants\[9\]. Its mechanism has not been understood yet. Thus it is important to study the Fe-stress molecular mechanism of maize in order to develop a new strategy to control plants with high-iron efficiency.

1 Materials and methods

(i) Plant material. Seeds of Yedan No.12 sensitive to Fe deficiency were surface-sterilized in 0.5%—1% (v/v) NaOCl, and germinated at 27°C for 36 h. Seedlings with 1-cm roots were transferred to the liquid aerated nutrient solution (Hoagland) without supplying Fe (-Fe treatment) at 25°C for 11—24 d. While Fe-deficiency symptoms occurred, the root tips including root hair region around 2 cm in length were obtained and stored at -80°C for use. At the same time, the control plants supplied with iron (1.4 mol/L +Fe treatment) were cultured under normal conditions.

(ii) Construction of cDNA library from Fe-stressed maize roots. A λZAP express cDNA library from Fe-deficient maize roots was constructed according to the protocol of ZAP Express™ cDNA Synthesis Kit and ZAP Express™ cDNA Gigapack Gold II Cloning Kit (Strategene, 1996). Above all, the reverse transcriptase (MMLV-RT)-mediated synthesis of the first-strand cDNAs was primed with the linker-primer containing

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\text{Xho I site: 5'GAGAGAGAGAGAGAGAGAACTAGTCTCGAGTTTTTTTTTTTTTTTTTTTTT3'}
\]

"GAGA" sequence \[
\text{Xho I Poly (dT)}
\]

The "GAGA" sequence can protect the Xho I restriction enzyme recognition site and the 18-base poly(dT) sequence. Moreover, 5-methyl dCTP instead of dCTP avoided digestion from restriction enzymes. The second-strand cDNAs were synthesized by RnasH and polymerase I at 16°C for 25 h, and then the uneven termini of the double-stranded cDNAs were nibbled or filled in with cloned DNA polymerase. EcoR I adapters were ligated to the blunt ends. Size fractionation was spun on a Sephacryl S-500 column, followed by Xho I digestion, then cDNAs with Xho I and EcoR I cohensived ends were recombined into Xho I and EcoR I sites of λZAP vectors. Having been packaged \textit{in vitro}, recombinant phage was transduced into XL1-Blue MRF² host strain. The cDNA library has been titered and the ratio of recombinants has been detected.

(iii) Differential hybridization screening. For differential screening of the iron-stressed cDNA library, Fe-sufficient(+ ) and Fe-deficient(−) cDNA probes were synthesized from (+) and (−) mRNA template by RT-PCR of random primers. After repeated clone screening using \textit{in situ} hybridization of bacteriophage plaques, the 6 plaques were selected from the cDNA library, and then were converted to the pBK-CMV phagmid by \textit{in vivo} rapid excision. The 6 clones were sequenced and homogeneously analyzed by TaKaRa Biotech (Japan).

(iv) Molecular hybridization. \textit{Fdr3} clone was digested with EcoR I/Xho I. Then \textit{Fdr3} fragment was recovered by Glassmilk either as probe of Northern blotting or as antigen of Western immunoblot analysis.

Total RNA from Fe-deficient maize roots was isolated using the acid guanidinium thiocyanate-phenol-chloroform method and then electrophoresed through agarose gel with 30 µg total RNA. Northern hybridization was analyzed by probe with labeled \textit{32P} by nick translation after transferring total RNA to nitrocellulose filters.