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

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Abstract To isolate Fe-deficient related (Fdr) genes, an expression cDNA library of 4.5×10⁶ pfu/µg 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 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 (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. 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, grasses respond to Fe-deficiency stress by enhancing the release of phytosiderophores (PS) which form chelate with Fe³⁺. Uptake of Fe³⁺-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.

Maize was classified as Strategy II due to PS (2’-deoxymugineic acid DMA) which was secreted from roots in Fe-deficient. Von Wiren et al. confirmed that iron inefficiency in the maize mutant 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 ⁵⁹Fe-[¹⁴C]-DMA by the Fe-efficient maize cultivator Alice and the Fe-inefficient mutant 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-infficiciency 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 (WF9) 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 dicotyledous 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 Cloing 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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