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The plasma membrane proteome of maize roots grown under low and high iron conditions☆

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ABSTRACT

Iron (Fe) homeostasis is essential for life and has been intensively investigated for dicots, while our knowledge for species in the Poaceae is fragmentary. This study presents the first proteome analysis (LC–MS/MS) of plasma membranes isolated from roots of 18-day old maize (*Zea mays* L.). Plants were grown under low and high Fe conditions in hydroponic culture. In total, 227 proteins were identified in control plants, whereas 204 proteins were identified in Fe deficient plants and 251 proteins in plants grown under high Fe conditions. Proteins were sorted by functional classes, and most of the identified proteins were classified as signaling proteins. A significant number of PM-bound redox proteins could be identified including quinone reductases, *heme* and copper-containing proteins. Most of these components were constitutive, and others could hint at an involvement of redox signaling and redox homeostasis by change in abundance. Energy metabolism and translation seem to be crucial in Fe homeostasis. The response to Fe deficiency includes proteins involved in development, whereas membrane remodeling and assembly and/or repair of Fe–S clusters is discussed for Fe toxicity. The general stress response appears to involve proteins related to oxidative stress, growth regulation, an increased rigidity and synthesis of cell walls and adaption of nutrient uptake and/or translocation.
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Abbreviations: ACS, acyl coenzyme a synthetase; AHA2, Arabidopsis ATPase/hydrogen-exporting ATPase 2; AIR12, auxin inducible in root cultures 12; BAK, brassinosteroid insensitive 1-associated receptor kinase; BR, brassinosteroid; COXII, cytochrome oxidase subunit II; DREPP, developmental regulated plasma membrane protein; DIR, dirigent-like proteins; EDTA, ethylenediaminetetraacetic acid; ER, endoplasmatic reticulum; FAD, flavin adenine dinucleotide; FAS, fasciclin; FMN, flavin mononucleotide; GDPD, glycerophosphodiester phospho-diesterase; GFP, green fluorescence protein; GPI, glycosylphosphatidylinositol; GPx, glutathione peroxidase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GTP, guanosine triphosphate; HSP, heat shock protein; H_2O_2, hydrogen peroxide; LC–MS/MS, liquid chromatography tandem mass spectrometry; MAPK, mitogen activated protein kinase; MARK, maize atypical receptor-like kinase; MGF, mascot generic file; MIP, major intrinsic protein; NA, nicotianamine; NPA, nucleosome assembly protein; pI, point isoelectric; PIP, plasma membrane intrinsic protein; PM, plasma membrane; PM0, plasma membranes of plants grown without iron; PM500, plasma membranes of plants grown with 500 μM iron; PM100, plasma membranes of control plants grown with 100 μM iron; Pti1, *Pseudomonas syringae* pv. *Tomato* interacting-like kinase; PTM, posttranslational modifications; ROS, reactive oxygen species; SAR1, secretion-associated and Ras-related protein 1; Sec21, gamma subunit, COP vesicles; SAM, S-adenosylmethionine; TIL, Temperature induced Lipocalin; YS1, yellow stripe 1.

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1. Introduction

Plants developed two strategies for Fe uptake [refs. 1–3]. Strategy I (dicots and non-grass monocots) is characterized by an enhanced proton extrusion which helps to solubilize Fe^{3+} from the soil and a subsequent reduction by a transmembrane Fe^{3+}-chelate reductase [3]. Iron (II) is then taken up by transporters like IRT1.

Grasses have a Fe uptake strategy distinct from all other plants, also called strategy II [refs. 2,3]. Grasses increase the production and secretion of phytosiderophores in response to Fe deficiency, and Fe^{3+}-phytosiderophore complexes are taken up by specific transporters. The genes for phytosiderophore synthesis have been isolated from several graminaceous plants [4–7] and high affinity Fe^{3+}-mugineic acid transporters (yellow stripe 1, YS1) have been isolated and characterized for maize (*Zea mays* L.), rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) roots [8–10].

Iron deficiency is a serious problem in worldwide crop production and results in reduced yields. Although Fe is ubiquitously present, its bioavailability is often low due to high pH values of the soil [3]. Iron-deficiency leads to oxidative stress, as some proteins of the respiratory chain are Fe-dependent and an under-representation of those proteins or an insufficient assembly with Fe may lead to an incomplete reduction of oxygen, resulting in the formation of superoxide radicals that are disproportionated by superoxide dismutases to hydrogen peroxide (H_2O_2).

Regardless of its importance, Fe has to be handled by sophisticated cellular control mechanisms because of its potential toxicity. Iron toxicity is well known to occur in wetland rice [11,12]. It can also take place in poorly aerated soils, for example through soil compaction, because under these conditions Fe^{3+} is reduced to Fe^{2+} which increases the bioavailability. Soil compaction occurs in agriculture and forestry due to the use of heavy machinery. The availability of Fe also increases in acid soils. As soil acidification also leads to other problems like Al toxicity or P deficiency, it is necessary to investigate the effects of Fe oversupply independently. Iron toxicity results in the formation of hydroxyl radicals produced by the Fenton reactions. Radicals cause oxidative stress and cell damage, but they are also involved in signaling processes [13].

Nowadays, involvement of plasma membrane (PM) redox systems in all these processes appears well established [14]. Changes in the abundance and/or composition of PM located oxidoreductases can therefore be expected if plants are exposed to high or low Fe concentrations and proteins which are involved in handling the problematic side effects of Fe should also be regulated.

So far, proteomic studies have focused on alterations in strategy I plants caused by Fe deficiency [15–20]. In the present study, a comparative nano liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis of solubilized PM was used to assess the regulation and function of PM-bound proteins in Fe uptake and oxidative stress. This is the first study which deals with Fe deficiency and toxicity simultaneously. Results indicate that proteins involved in transport and signaling were the major components. Changes were observed for these and other functional classes of proteins in plants that were grown under low and high concentrations of Fe. Possible functions of proteins, having significant change in abundance, are discussed.

2. Materials and methods

2.1. Plant growth

Caryopses of *Z. mays* (cv. Gelber Badischer Landmais, Saatenunion, Hannover, Germany) were soaked for 6 h and placed for germination between two layers of wet growth paper in the dark for four days at 25 °C. Afterwards, seedlings were transferred to 9 L plastic boxes containing nutrient solution pH 5.5 prepared according to [21] with 0 μM, 100 μM or 500 μM Fe(III)EDTA. The solution was changed after seven days, and the plants were harvested after two weeks of growth into the nutrient solution, so that they grew finally for 18 days.

2.2. Preparation of plasma membrane

Whole roots (60–100 g, approximately 240 plants) were homogenized in the presence of 250 mM sucrose, 50 mM HEPES/KOH pH 7.0, 1 mM Na_2(EDTA), 1 mM dithiothreitol and 1% of insoluble polyvinylpyrrolidone (Sigma, Deisendorf, Germany). The homogenate was pressed through a nylon net (125 μm mesh; Hydrobios, Kiel, Germany), supplemented with 1 mM phenylmethylsulfonyl fluoride and centrifuged for 10 min at 10,000 *g* to remove organelles and cell debris. The supernatant was then centrifuged for 30 min at 50,000 *g* to sediment the microsomal fraction, which is considered to consist of vesicles of the different membranes.

Plasma membranes were prepared using aqueous two phase partitioning according to [22], with the exception that the lower phases were not reextracted by a fresh polyethylene glycol upper phase. Vesicles were washed three times in a buffer containing 150 mM KCl, 50 mM HEPES/KOH pH 7.0, 1 mM Na_2(EDTA) and 0.01% Triton X-100. In order to remove soluble proteins that are trapped into the vesicles, they were inverted by freezing and thawing between the washing steps. Finally, the PM preparation was resuspended in 50 mM HEPES/KOH pH 7.0 containing 0.25 M sucrose and stored at −76 °C.

Purity of PM preparations was estimated using a plant cell compartment antibody marker set (Agrisera, Vännäs, Sweden). Polyclonal antibodies against H^+-ATPase (PM), V-ATPase (tonoplast), SAR1 (endoplasmic reticulum, ER), Sec21 (golgi apparatus), and cytochrome c oxidase (COXII, inner mitochondrial membrane) were used for Western-blot analysis as recommended by the distributor. For each marker, 5 μg protein was loaded on the SDS-PAGE gel. Microsomal fractions were used as positive controls.

2.3. Sample preparation for mass spectrometry

Three PM preparation from each Fe concentration with 2 technical replicates were analyzed. Proteins were solubilized in 125 mM Tris/HCl, pH 6,8, with 2% SDS in the presence of 6 M urea and 5% mercaptoethanol for 2 h at room temperature. The protein concentration was 1 μg/μL. The mixture was

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