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Research article

Low iron availability and phenolic metabolism in a wild plant species (*Parietaria judaica* L.)

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ABSTRACT

Plant phenolics encompass a wide range of aromatic compounds and functions mainly related to abiotic and biotic environmental responses. In calcareous soils, the presence of bicarbonate and a high pH cause a decrease in iron (Fe) bioavailability leading to crop yield losses both qualitatively and quantitatively. High increases in phenolics were reported in roots and root exudates as a consequence of decreased Fe bioavailability suggesting their role in chelation and reduction of inorganic Fe(III) contributing to the mobilization of Fe oxides in soil and plant apoplast. Shikimate pathway represents the main pathway to provide aromatic precursors for the synthesis of phenylpropanoids and constitutes a link between primary and secondary metabolism. Thus the increased level of phenolics suggests a metabolic shift of carbon skeletons from primary to secondary metabolism. *Parietaria judaica*, a spontaneous plant well adapted to calcareous environments, demonstrates a high metabolic flexibility in response to Fe starvation. Plants grown under low Fe availability conditions showed a strong accumulation of phenolics in roots as well as an improved secretion of root exudates. *P. judaica* exhibits enhanced enzymatic activities of the shikimate pathway. Furthermore, the non-oxidative pentose phosphate pathway, through the transketolase activity supplies erythrose-4-phosphate, is strongly activated. These data may indicate a metabolic rearrangement modifying the allocation of carbon skeletons between primary and secondary metabolism and the activation of a nonoxidative way to overcome a mitochondrial impairment. We suggest that high content of phenolics in *P. judaica* play a crucial role in its adaptive strategy to cope with low Fe availability.

1. Introduction

Iron (Fe) is an essential element for life. Due to its redox characteristics Fe plays an important role in cell metabolism. In particular, in mitochondrial and photosynthetic electron transport chains it is directly involved in the energy-yielding processes. Iron is one of the most abundant elements in the Earth's crust but, in spite of this, in oxygenated environments it is found in ferric forms (FeIII), mainly oxides and hydroxides, that are very stable and scarcely soluble at neutral pH, reaching a concentration far below that required for optimal plant growth [1]. In alkaline soils, mainly calcareous ones, Fe solubility dramatically decreases and it is not readily taken up by the plant, leading to consistent crop yield losses both qualitatively and quantitatively. Accordingly, plants have evolved two different strategies to face Fe acquisition: a scavenging strategy and a mining one. Scavenging strategy concerns those mechanisms implemented for soil exploration and results in changes in root growth, morphology and architecture, as well as in increased root hairs density. Mining strategy is aimed at mobilizing nonbioavailable forms of Fe and includes three chemical processes, reduction, acidification and chelation. Obviously these two strategies operate together.

Dicots and nonPoaceae monocots have evolved a reduction based mining mechanism (Strategy I) localized at the root cell membrane to cope with low Fe availability [2]. Strategy I includes: a Fe specific mechanism of reduction via a transmembrane Fe(III) Chelate Reductase (FC-R), which reduces ferric to ferrous ions that are transported inside the cell by a Fe regulated transporter (IRT); and a nonspecific mechanism of protonation by means of the H^+-ATPase that extrudes protons acidifying the rhizosphere and consequently increasing Fe solubility [3]. In addition plants exude a large variety of substances even further acidifying the rhizosphere and acting as organic complexing agents that can contribute to mobilize minerals in soil solution [4]. In many species phenolics

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have been recognized as an important component of root exudates [5]. Phenolics are a wide group of hydroxylated aromatic compounds found only in microorganisms and plants. They are secondary metabolites that show a huge diversity of structures, from rather simple structures, as phenolic acids, through polyphenols, for instance flavonoids, that comprise several groups, to polymeric compounds such as tannins or lignin. Phenolics play multiple chemical and biological functions in plants mainly related with adaptation to environmental changes [6]. They represent a clear example of metabolic plasticity as plants are able to respond to external stresses rapidly inducing their biosynthesis in a reversible way [7]. Countless works reported the increase of phenolic content in plants tissues, roots and root exudates as a response to different biotic and abiotic stresses [8–10]. Among these it has been registered an induction of phenolics secretion by roots under Fe-deficient conditions [8,11,12]. A multiple role of phenolics as mediators in Fe acquisition has been suggested. Phenolics could operate in three ways: by directly improving Fe solubility mainly due to their reductant and chelating properties; by mediating the reutilization of root apoplastic Fe and by their allelopathic activity influencing the rhizosphere microbial communities to produce siderophores and auxin [13]. Hence phenolics can indeed affect nutrient availability [10].

Several studies have underlined that under abiotic stress plants have to face the trade-off in carbon skeletons allocation between growth, maintenance and defence [14,15].

The shikimate pathway is the common route to provide aromatic precursors for further secondary metabolites. It also represents a link between primary and aromatic secondary metabolism. It was estimated that 60% of total plant biomass is composed of molecules that have traversed the shikimate pathway [14]. Successfully coping with a stress conditions such as Fe deficiency presupposes a fine metabolic rearrangement arising from contrasting physiological requirements. In fact, plants have to manage, on one hand, with an increased demand of ATP and NADH required for Fe acquisition by Strategy I mechanisms concomitant to the impaired mitochondrial transduction of energy and, on the other hand, the carbon skeleton flux shifting toward the production of secondary metabolites.

Parietaria judaica L. (Urticaceae) is a wild perennial and sinantropic dicot that from a nutritional point of view behave as an “indifferent” plant. It grows in both acidic and alkaline soils but it usually represents the most widespread flora in highly calcareous and hostile environments such as wall cracks exposed to the sun. In these calcareous environments *P. judaica* does not show any symptom of chlorosis which is the primary symptom of Fe deficiency. Previous data showed that *P. judaica* activates all Strategy I mechanisms as a response to Fe deficiency but the degree of activation does not explain the efficiency through which this species adapts to low Fe bioavailability. Significant root morphological modifications have also been observed in Fe-deficient growth conditions [16,17].

High phenolic content is a characteristic of *P. judaica* species; nevertheless it has been observed a significant increase of them in roots and exudates when subject to Fe deficiency [17]. In this work we grew *P. judaica* in different direct or induced Fe-deficient hydroponic conditions: a control full nutrient solution (+Fe), a minus Fe solution (−Fe) deprived of Fe, an alkaline full nutrient supplemented with $NaHCO_3$ and $CaCO_3$ that brings the pH to 8.3 and mimics a calcareous environment, an alkaline full nutrient solution buffered with Tricine (Tric) adjusted to pH 8.3. Tric treatment was performed in order to discriminate the response caused only by the pH effect from that due to the presence of the bicarbonate ion. We focused our attention on the mechanisms through which *P. judaica* shifts carbon skeletons from primary to secondary metabolism. We also investigated how primary metabolism rearranges to supply the substrates for shikimate pathway (erythrose 4-phosphate, E4P and phospho*enol*pyruvate, PEP) considering that under Fe deficiency there is a greater demand of energy and reducing power as a consequence of the activation of proton extrusion and Fe(III) reduction, and on the other hand an impaired mitochondrial activity.

2. Results

2.1. Root modifications

Previous works have reported modifications in root morphology in *P. judaica* grown in Fe deficiency conditions pointing out the presence of an increased number of secondary roots as well as the onset of clusters of short and thick rootlets [16]. After 7-d treatment we observed results similar to those obtained before. In fact in all treatments we found a shorter but more branched radical system in terms of both adventitious and lateral roots with respect to control. Anyway, it can be noted a different branching pattern in −Fe, Bic and Tric treatment (Fig. 1). −Fe roots develop a lot of very short sketches of lateral roots (Fig. 1B). In particular, we observed a more similar root system in Bic and Tric plants even though shoots show a quite different response. In fact, Bic shoots exhibit a slowdown in growth and more chlorotic leaves respect to Tric (Fig. 1C and D).

2.2. Phenolic compounds in roots and root exudates

Phenolic concentration was measured in roots and exudates of 7-d-old plants. The content of soluble phenolics found in root extracts of all stress treatments shows higher values increased by 40–50% respect to the control but no significant differences were found between −Fe, Bic and Tric conditions. Phenolic content obtained by methanolic extraction also shows an increase in polar phenolics in comparison with control of nearly 60% for −Fe and Tric treatments while in Bic a remarkable increase (nearly 4-fold compared to the control and more than 1-fold respect to the other treatments) was found. Furthermore, it can be noted that in Bic condition the content of polar phenolics is much higher than that of soluble ones (Fig. 2B). Phenolic content found in field samples collected from alkaline substrates are comparable to those measured in samples grown in hydroponic conditions at high bicarbonate content (Fig. 2A). In root exudates (Fig. 2C) a noteworthy increase in phenolics in all stress conditions (5- to 8-fold) with respect to control was detected; while Bic and Tric conditions show comparable phenolic content in exudates, −Fe plants extrude a significant higher amount.

2.3. Responses of primary metabolism

Fe deficiency induces Strategy I mechanisms to facilitate Fe acquisition, among these FC-R and H^+-ATPase activities, which require a constant production of energetic substrates. As glucose is the main and most immediate source of energy in plant metabolism, some key enzymatic activities belonging to the glycolytic pathway have been determined.

Table 1 shows the results obtained assaying the hexokinase (HK), glyceraldehide 3-phosphate dehydrogenase (GAPDH) and phospho*enol*pyruvate carboxylase (PEPC) activities on root soluble extracts.

HK catalyzes the first step of glycolysis and has been shown to be involved in a complex regulatory mechanism related with shoot and root growth [18]. GAPDH is the first enzyme of the so called energy harvesting glycolytic steps. HK and GAPDH catalyze reactions that generate H^+ and play an important role both in