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Research article

Constitutive expression of a barley Fe phytosiderophore transporter increases alkaline soil tolerance and results in iron partitioning between vegetative and storage tissues under stress

Sonia Gómez-Galera [a], Duraialagaraja Sudhakar [b], Ana M. Pelacho [c], Teresa Capell [a], Paul Christou [a,d,*]

[a] Department of Plant Production and Forestry Science, ETSEA, University of Lleida-CRA, Av. Alcalde Rovira Roure 191, E-25198 Lleida, Spain
[b] Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore 641 003, India
[c] Department of Hortofruticulture, Botany and Gardening, ETSEA, University of Lleida-CRA, Av. Alcalde Rovira Roure 191, E-25198 Lleida, Spain
[d] ICREA, Passeig Lluís Companys 23, 08010 Barcelona, Spain

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ABSTRACT

Cereals have evolved chelation systems to mobilize insoluble iron in the soil, but in rice this process is rather inefficient, making the crop highly susceptible to alkaline soils. We therefore engineered rice to express the barley iron-phytosiderophore transporter (HvYS1), which enables barley plants to take up iron from alkaline soils. A representative transgenic rice line was grown in standard (pH 5.5) or alkaline soil (pH 8.5) to evaluate alkaline tolerance and iron mobilization. Transgenic plants developed secondary tillers and set seeds when grown in standard soil although iron concentration remained similar in leaves and seeds compared to wild type. However, when grown in alkaline soil transgenic plants exhibited enhanced growth, yield and iron concentration in leaves compared to the wild type plants which were severely stunted. Transgenic plants took up iron more efficiently from alkaline soil compared to wild type, indicating an enhanced capacity to increase iron mobility *ex situ*. Interestingly, all the additional iron accumulated in vegetative tissues, i.e. there was no difference in iron concentration in the seeds of wild type and transgenic plants. Our data suggest that iron uptake from the rhizosphere can be enhanced through expression of *HvYS1* and confirm the operation of a partitioning mechanism that diverts iron to leaves rather than seeds, under stress.

1. Introduction

Many plants grow poorly in alkaline soils, which account for ~30% of the world's arable land [21]. One of the problems of alkaline soils is that they limit iron uptake. Plants need iron to synthesize chlorophyll and to carry out photosynthesis, so iron deficiency results in chlorosis, poor growth and reduced yields. Iron in alkaline soils is present mostly as Fe^{3+}, which is insoluble and therefore inaccessible to the plants. This limitation cannot easily be overcome using Fe^{2+} fertilizers because the soluble iron is rapidly converted into Fe^{3+} *in situ* [10]. Crops growing in alkaline soils also fail to accumulate iron in edible organs causing nutrient deficiency in humans [10].

Plants have evolved different mechanisms to overcome iron limitation in alkaline soils [2,9]. Non-graminaceous species release protons into the rhizosphere to increase the solubility of Fe^{3+} by acidification, and the Fe^{3+} is then reduced to Fe^{2+} by a membrane-bound ferric reductase oxidase [25]. This allows the uptake of iron into the root cells through iron-regulated transporter 1 (IRT1) [9]. Iron absorption in this manner is known as strategy I. Graminaceous plants use a mechanism based on iron chelation, which involves the secretion of molecules known as phytosiderophores (PS) and the subsequent absorption of PS–Fe^{3+} complexes [14,21]. This is known as strategy II, and it is considered more efficient than strategy I.

In graminaceous crops, alkaline tolerance correlates with the amount of PS secreted into the soil, and cereals can be ranked in the following order starting with the most tolerant: barley/wheat > oat/rye > maize/sorghum > rice [27]. Among the staple cereal crops, rice is therefore the most susceptible to iron deficiency in alkaline soils. Susceptibility of rice to such alkaline conditions is

Abbreviations: HvYS1, barley iron-phytosiderophore transporter; ICP-MS, inductively coupled plasma mass spectrometry; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NA, nicotianamine; PS, phytosiderophores; Ubi-1, maize ubiquitin-1 promoter.

* Corresponding author. Department of Plant Production and Forestry Science, ETSEA, University of Lleida-CRA, Av. Alcalde Rovira Roure 191, E-25198 Lleida, Spain. Tel.: +34 973702831; fax: +34 973238264.
E-mail address: christou@pvcf.udl.es (P. Christou).

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thought to occur due to the low amounts of PS that are secreted by the roots. Rice is the main source of calories for nearly half the world's population, with 40% and 70% of individuals relying solely on rice in Asia and Africa, respectively [44]. Therefore rice is arguably the most important target for genetic engineering strategies to address alkaline tolerance.

Genetic engineering has been used to introduce genes encoding iron transporters, iron reductases and enzymes involved in PS biosynthesis into plants, and this has enhanced the uptake and accumulation of iron in several crops, especially under iron-limiting conditions [2,9,10,45]. 'Strategy I' transgenic rice plants have been engineered to express yeast iron reductases [13], as well as transcription factors controlling the expression of genes induced by iron deficiency, such as IDEF1 and OsIRO2 [20,28,29]. 'Strategy II' transgenic rice plants have also been reported, expressing barley genes encoding enzymes involved in PS biosynthesis such as IDS3 (2-oxoglutarate-dependent dioxygenase; IDS = iron deficiency specific), nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT). Rice plants expressing these genes produced and secreted more PS than wild type plants and were more tolerant to alkaline soils both in the laboratory [12,17,18,22] and under iron-limiting conditions in the field [36]. In addition, seeds from transgenic rice plants expressing IDS3, NAS2 and OsIRO2 contained more iron than their wild type counterparts [15,17,18,28,36].

In strategy II plants, iron-phytosiderophore transporters are required to import PS–Fe^{3+} complexes into root cells, and these are encoded by *YS1* genes, named after the yellow stripe phenotype observed when the first such gene was discovered in maize [7]. ZmYS1 transports several ions as PS complexes, including Fe^{3+}, Fe^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+} and Cd^{2+}. In addition, ZmYS1 also transports the nicotianamine complexes Fe^{2+}–NA and Ni^{2+}–NA [27,34]. Murata et al. [27] cloned and characterized the corresponding barley gene (*HvYS1*) whose expression was shown to be root-specific and inducible under iron-limiting conditions. Interestingly, although HvYS1 is closely related to ZmYS1, the barley protein is specific for PS–Fe^{3+} complexes [27].

We reasoned that a heterologous PS–Fe^{3+} transporter expressed in rice would increase the accumulation of iron under the limiting conditions imposed by alkaline soils, and that HvYS1 would be an ideal candidate because its specificity would prevent the displacement of iron by less desirable minerals. We therefore produced transgenic rice plants expressing the transgene strongly and consistently, and expanded one line to test T1 plants for alkaline tolerance and iron accumulation. Although the transgenic plants were more tolerant to alkaline soils than controls and took up more iron under limiting conditions, additional iron accumulated in vegetative tissues rather than seeds, suggesting the operation of a partitioning mechanism for iron under stress.

2. Results

2.1. Identification of transgenic rice lines expressing HvYS1

Bombarded rice embryos were regenerated under hygromycin selection leading to the recovery of 16 independent transgenic lines, each of which was evaluated by northern blot analysis to detect *HvYS1* steady state mRNA in the roots and leaves. Eight lines showed strong and consistent *HvYS1* expression in both tissues (Fig. 1). Although all the lines produced seeds, line L16 was selected for analysis because it produced enough seeds for the statistically-relevant testing of next generation plants under standard and alkaline soil conditions. The seeds were germinated on medium containing hygromycin to screen out negative segregants, and ten plantlets each were transferred to pots containing standard soil facilitating iron uptake (pH 5.5) and alkaline soil with low iron mobility (pH 8.5).

2.2. Transgene expression in L16 T1 plants

Transgene expression in T1 plants was determined by northern blot using mRNA extracted from the leaves of two plants from each group at the beginning and end of the 8 week treatment period. As shown in Fig. 2, high-level transgene expression was observed under both treatment regimens at both sampling time points, whereas no expression was observed in wild type controls as expected. These results confirmed that the transgene is constitutively expressed in T1 plants and is not influenced by the different soil conditions.

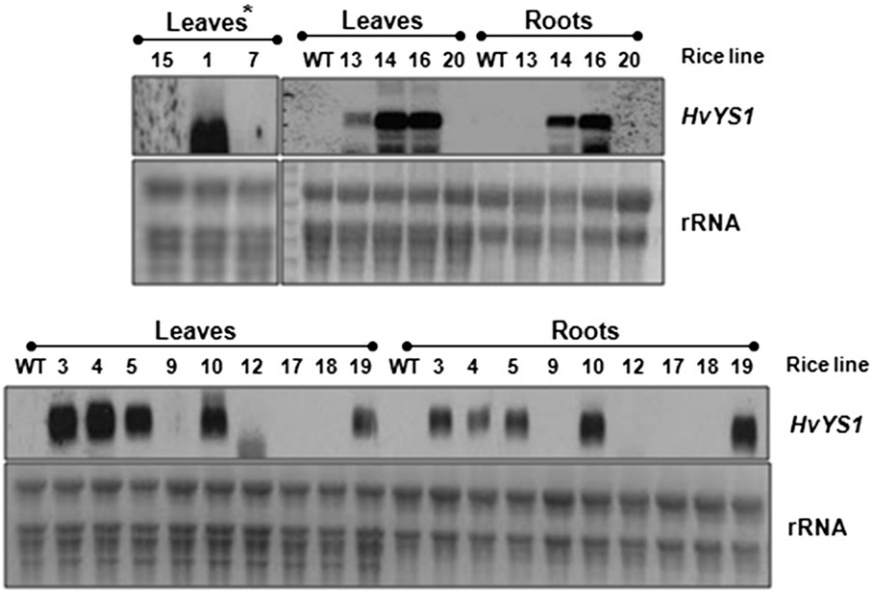

Fig. 1. Northern blot analysis of *HvYS1* expression in the leaves and roots of 16 independent rice transformants. (*) There was insufficient material for mRNA analysis in the roots of these three lines. Eight of the 16 rice transformants showed *HvYS1* expression. WT, wild type control. Numbers indicate independent transgenic lines.