

RESEARCH PAPER

# Further characterization of ferric–phytosiderophore transporters ZmYS1 and HvYS1 in maize and barley

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## Abstract

Roots of some gramineous plants secrete phytosiderophores in response to iron deficiency and take up Fe as a ferric–phytosiderophore complex through the transporter YS1 (Yellow Stripe 1). Here, this transporter in maize (ZmYS1) and barley (HvYS1) was further characterized and compared in terms of expression pattern, diurnal change, and tissue-type specificity of localization. The expression of HvYS1 was specifically induced by Fe deficiency only in barley roots, and increased with the progress of Fe deficiency, whereas ZmYS1 was expressed in maize in the leaf blades and sheaths, crown, and seminal roots, but not in the hypocotyl. HvYS1 expression was not induced by any other metal deficiency. Furthermore, in maize leaf blades, the expression was higher in the young leaf blades showing severe chlorosis than in the old leaf blades showing no chlorosis. The expression of HvYS1 showed a distinct diurnal rhythm, reaching a maximum before the onset of phytosiderophore secretion. In contrast, ZmYS1 did not show such a rhythm in expression. Immunostaining showed that ZmYS1 was localized in the epidermal cells of both crown and lateral roots, with a polar localization at the distal side of the epidermal cells. In maize leaves, ZmYS1 was localized in mesophyll cells, but not epidermal cells. These differences in gene expression pattern and tissue-type specificity of localization suggest that HvYS1 is only involved in primary Fe acquisition by barley roots, whereas ZmYS1 is involved in both primary Fe acquisition and intracellular transport of iron and other metals in maize.

**Key words:** Barley, expression pattern, iron, localization, maize, phytosiderophore, transporter.

## Introduction

Some gramineous plants such as barley and wheat have a distinct strategy (Strategy II) for iron (Fe) acquisition, which is different from that of non-grass species (Marschner and Römheld, 1994; Curie and Briat, 2003; Walker and Connolly, 2008). Iron acquisition by gramineous plants includes (i) biosynthesis of phytosiderophores (mugineic acids) inside the roots; (ii) secretion of phytosiderophores to the rhizosphere; (iii) solubilization of insoluble iron in soils by chelation of phytosiderophores; and (iv) uptake of the ferric–phytosiderophore complex by the roots (Ma, 2005). It has been demonstrated that the last step is mediated through a transporter for the ferric–phytosiderophore complex. The first gene (*YS1*, *Yellow Stripe 1*) encoding the ferric–phytosiderophore transporter was identified in maize (Curie *et al.*, 2001). YS1 belongs to the

oligopeptide transporter (OPT) family (Yen *et al.*, 2001), but quite distantly as it shares only ~10% similarity and does not contain the OPT consensus domain (Koh *et al.*, 2002). There are eight and 19 homologues (YSL, Yellow Stripe-like proteins) of YS1 in the *Arabidopsis* and rice genome, respectively (Curie *et al.*, 2001; Jean *et al.*, 2005). Some homologues have also been identified in barley (Murata *et al.*, 2006) and in a dicot species, *Thlaspi caerulescens* (Gendre *et al.*, 2007). The presence of YSL in non-grass species suggests that YSLs are involved not only in iron acquisition, but also in the internal transport of iron.

Previous studies suggest that there are at least two distinct groups of YSLs based on their expression patterns and transport substrates. One group transports the Fe(III)–phytosiderophore complex from the rhizosphere to the root

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cells, and includes ZmYS1, HvYS1 from barley, and OsYSL15 from rice (Curie *et al.*, 2001; Murata *et al.*, 2006; Inoue *et al.*, 2009). The other group is responsible for the intracellular transport of iron and other metals complexed with nicotianamine (NA), and includes OsYSL2, AtYSL1, 2, 3, and TcYSL3. In contrast to the known transporters for ferric-phytosiderophore in roots (e.g. ZmYS1, HvYS1, and OsYSL15), transporters such as OsYSL2 from rice and AtYSL2 from *Arabidopsis* did not transport the Fe(III)-phytosiderophore complex in yeast and/or *Xenopus* oocyte assay (DiDonato *et al.*, 2004; Koike *et al.*, 2004). Instead, OsYSL2 transports Fe(II)-NA and Mn-NA complexes (Koike *et al.*, 2004), and AtYSL2 transports Fe(II)-NA and Cu-NA complexes in yeast (DiDonato *et al.*, 2004), although contrasting results also have been reported (Schaaf *et al.*, 2005). OsYSL2 is expressed in the companion cells of leaf blades and sheaths, and has been suggested to be involved in phloem transport of Fe and Mn, while AtYSL2 is expressed in many cell types in both roots and shoots and is suggested to be involved in the lateral movement of metals in the vasculature and in Fe and Zn homeostasis (DiDonato *et al.*, 2004; Schaaf *et al.*, 2005). The transport substrates for AtYSL1 and AtYSL3 have not been identified, but analysis with single and double mutants suggested that these genes are responsible for mobilization of micronutrients such as Mn, Zn, Cu, and Fe from leaves and for loading of Fe-NA into the seeds (Jean *et al.*, 2005; Waters *et al.*, 2006).

HvYS1 is the closest homologue of ZmYS1, sharing 72.7% identity and 95.0% similarity, but they have different expression patterns and transport substrates. HvYS1 is only expressed in the roots (Murata *et al.*, 2006), whereas ZmYS1 is expressed in both roots and shoots (Curie *et al.*, 2001). Both transporters are greatly up-regulated by Fe deficiency. Furthermore, in contrast to HvYS1, which transports the ferric-phytosiderophore complex only (Murata *et al.*, 2006), ZmYS1 also transports other substrates including zinc-, copper-, and nickel-phytosiderophore complexes, and nickel, Fe(II), and Fe(III) complexes with NA in yeast and *Xenopus* oocytes (Roberts *et al.*, 2004; Schaaf *et al.*, 2004). These results suggest that ZmYS1 and HvYS1 may have different roles in iron and metal transport. However, since HvYS1 and ZmYS1 were characterized by different groups previously, results may not be comparable. For example, the localization of HvYS1 activity has been examined, but that of ZmYS1 is unknown. In the present study, to make ZmYS1 and HvYS1 comparable, they were further characterized in terms of expression pattern and tissue localization.

## Materials and methods

### *Plant materials and growth condition*

Seeds of barley (*Hordeum vulgare* L., cv. Morex) and maize (*Zea mays* L., cv. B73) were soaked in deionized water for 2 h, and then incubated overnight on moist paper in the

dark at 20°C for barley or 25°C for maize. Germinated seeds were transferred to a net floated on a continuously aerated solution containing 0.5 mM CaCl<sub>2</sub> (pH 5.6) in the dark. Seedlings were then transferred into the aerated 1/5 Hoagland's solution (pH 6.0) containing 1 mM KNO<sub>3</sub>, 1 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.4 mM MgSO<sub>4</sub>, and 0.2 mM KH<sub>2</sub>PO<sub>4</sub>, and the micronutrients comprising 20 μM Fe-EDTA, 3 μM H<sub>3</sub>BO<sub>3</sub>, 1.0 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 0.5 μM MnCl<sub>2</sub>, 0.4 μM ZnSO<sub>4</sub>, and 0.2 μM CuSO<sub>4</sub>. The nutrient solution was changed once every 3 d. At least three biological replicates were conducted for all experiments.

### *Time-dependent and metal-responsive expression of HvYS1*

Barley seedlings (12-d-old) were subjected to a nutrient solution without Fe in a growth chamber (22°C day/18°C night, 12 h/12 h). On days 0, 1, 2, 4, and 7, roots and shoots were sampled and subjected to RNA extraction as described below. The expression of *HvYS1* in different tissues was examined by real-time RT-PCR as described below. To investigate the response of *HvYS1* to different metal deficiency, barley seedlings (12-d-old) were grown in a nutrient solution without either Fe, Mn, Cu, or Zn for 7 d. Roots and shoots were then sampled and subjected to RNA extraction and quantitative analysis of gene expression as described below.

### *Tissue-specific expression of ZmYS1*

Maize seedlings (14-d-old) were cultivated in a nutrient solution without Fe in a glasshouse. After 5 d the leaf blade, leaf sheath, hypocotyl, crown roots, and seminal roots were separated and used for RNA extraction. The expression of *ZmYS1* in different tissues was examined by RT-PCR as described below.

To investigate the expression level of *ZmYS1* in different leaf blades with different Fe status, the maize seedlings were first grown in a nutrient solution with Fe in a glasshouse. When the second leaf was fully expanded, the seedlings (7-d-old) were transferred to a nutrient solution without Fe. After 4 d, when the fourth leaf was expanded, each leaf blade was sampled and used for RNA extraction and quantitative analysis of *ZmYS1*. The SPAD value of each leaf blade was measured with the chlorophyll meter, SPAD-502 (Konica Minolta, Tokyo, Japan).

### *Time-dependent expression of ZmYS1 in different tissues*

To investigate time-dependent change of *ZmYS1* expression in different tissues, the maize seedlings were pre-cultured with Fe for 10 d in a glasshouse. The seedlings were then grown in a nutrient solution without Fe for 0, 1, 2, 4, or 7 d. The youngest leaf blade and seminal roots were harvested separately, and used for RNA extraction and quantitative analysis of *ZmYS1* as described below.

### Collection and quantitative analysis of phytosiderophore

The root exudates were collected from Fe-deficient barley and maize plants. On the day before exudate collection, roots were washed with deionized water twice, and then placed in 1.2 l of deionized water. Collection of the root exudates was started before sunrise (~7:00 h for barley and 5:00 h for maize) in a glasshouse. At 3 h intervals over the 24 h period, the exudate solution was removed and roots were transferred to fresh deionized water. The experiments were performed in November for barley and in July for maize. The root exudates collected were immediately passed through a cation-exchange column (16 mm×14 cm) filled with Amberlite IR 120B (H<sup>+</sup> form, Organo Co., Tokyo, Japan) and eluted with 2 M NH<sub>4</sub>OH (Ma *et al.*, 2003). The eluates were concentrated by using a rotary evaporator at 40°C. After the residues were dissolved with 1 ml of distilled water, the amounts of phytosiderophores were determined by measuring their iron-solubilizing capacity according to Takagi (1976), with some modifications (Ma *et al.*, 2003), for barley, and by HPLC according to Ueno *et al.* (2007) for maize.

At each sampling time point, the roots and the shoots were also sampled and frozen in liquid nitrogen for RNA extraction as described below.

### RNA extraction and gene expression quantification

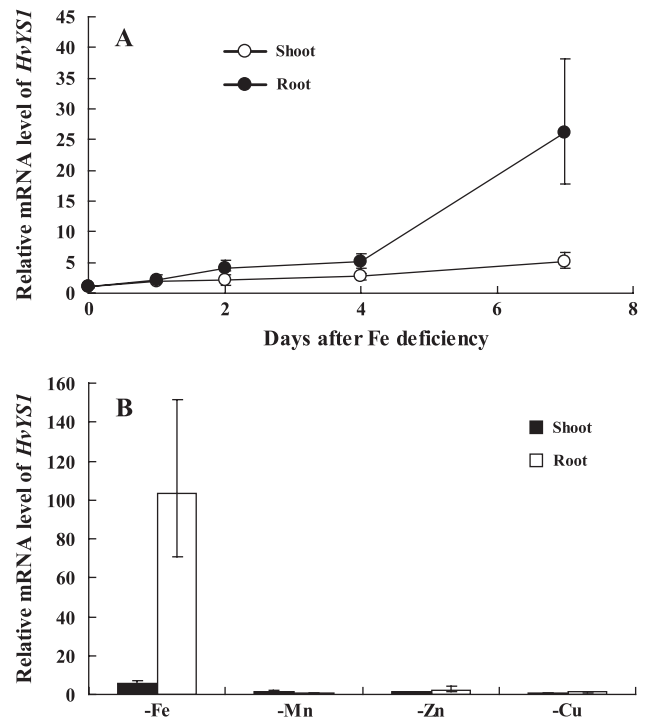
Total RNA was extracted from frozen plant samples by using an RNeasy plant mini kit (Qiagen) and then converted to cDNA followed by DNase I treatment using the protocol supplied by the manufacturers of SuperScript II (Invitrogen). Specific cDNAs were amplified by SYBR pre-mix EX Taq™ (Takara) and real-time RT-PCR (ABI Prism 7500, Applied Biosystems) with the following primer sets; 5'-AAAAAATGCGGACGACTGT-3' and 5'-AGGCATAACCAGCGTATGCC-3' for *HvYS1*, 5'-GTTTCAGTTTCCTACGTTTCGGTC-3' and 5'-TCTCCACCGTTTGCCACTCTG-3' for *ZmYS1*, and 5'-GACTCTGGTGATGGTGTGTCAGC-3' and 5'-GGCTGGAAGAGGACCTCAGG-3' for *Actin* as an internal standard.

### Immunostaining of *ZmYS1*

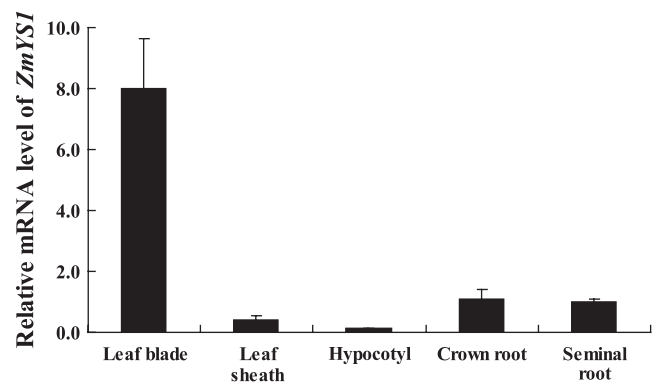
Antibody against *ZmYS1* was prepared by immunizing rabbits with a synthetic peptide C-DEMAALDDLQRDEI (positions 377–390 of *ZmYS1*). *ZmYS1* immunostaining was performed in both seminal and crown roots and leaf blades of Fe-deficient maize as described previously (Murata *et al.*, 2006). Tissues were sliced into 100 μm thick sections for immunostaining. The Axio Imager with Apotome (Carl Zeiss) was used for observation.

### Statistical analysis

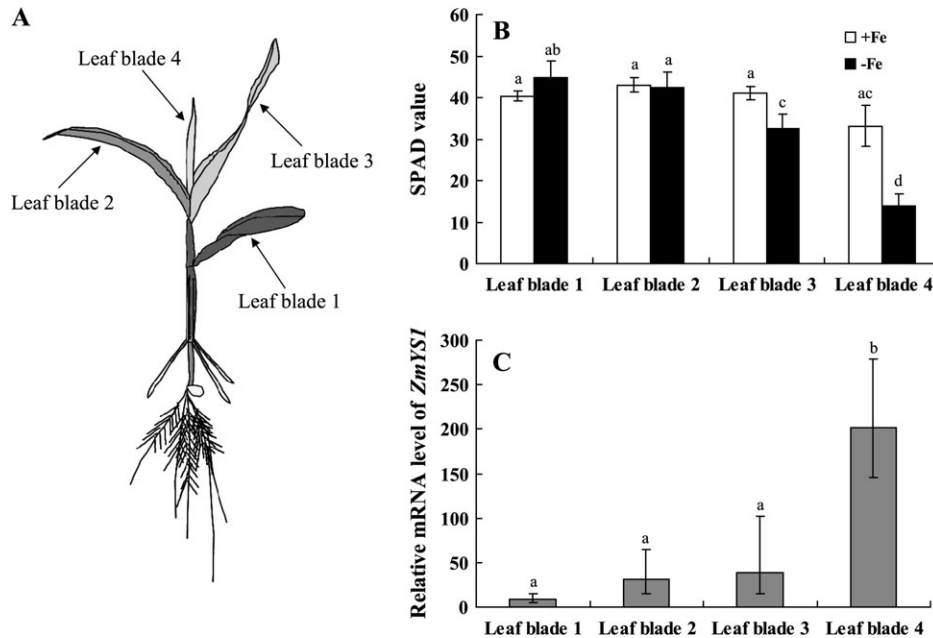
Analysis of variance (ANOVA) was performed on all data sets.



**Fig. 1.** Expression of *HvYS1* in the roots and shoots of barley. (A) Time-dependent expression of *HvYS1*. Barley seedlings were grown in Fe-free nutrient solution and sampled on different days. (B) Response of *HvYS1* to deficiency of different metals. Barley seedlings were grown in a nutrient solution without Fe, Mn, Cu, or Zn for 7 d. The expression level of *HvYS1* on different days or in different tissues was determined by quantitative RT-PCR. The mRNA expression level as relative mRNA levels was, respectively, normalized based on the expression level of different tissues of Fe-sufficient plants. The *Actin* mRNA level was used as an internal control. Data are means ±SD ( $n=3$ ).



**Fig. 2.** Tissue-specific expression of *ZmYS1*. Maize seedlings were grown in Fe-free nutrient solution for 14 d and then separated into different tissues. The expression level of *ZmYS1* in different tissues was determined by quantitative RT-PCR. The mRNA expression level as relative mRNA levels was normalized based on the expression level of the seminal roots of Fe-deficient plants. The *Actin* mRNA level was used as an internal control. Data are means ±SD ( $n=3$ ).



**Fig. 3.** Effect of Fe status on the expression level of *ZmYS1* in different leaf blades. Maize seedlings were grown in a nutrient solution with Fe for 7 d and then in a solution without Fe for 4 d. (A) A maize plant showing Fe deficiency symptoms in the young leaves. (B) SPAD value of different leaf blades with or without Fe supply. (C) Expression of *ZmYS1* in different leaf blades. The expression level of *ZmYS1* in different tissues was determined by quantitative RT-PCR. The mRNA levels are relative to those of the +Fe plants. Different letters indicate a significant difference at  $P < 0.05$ . Data are means  $\pm$  SD ( $n=3$ ).

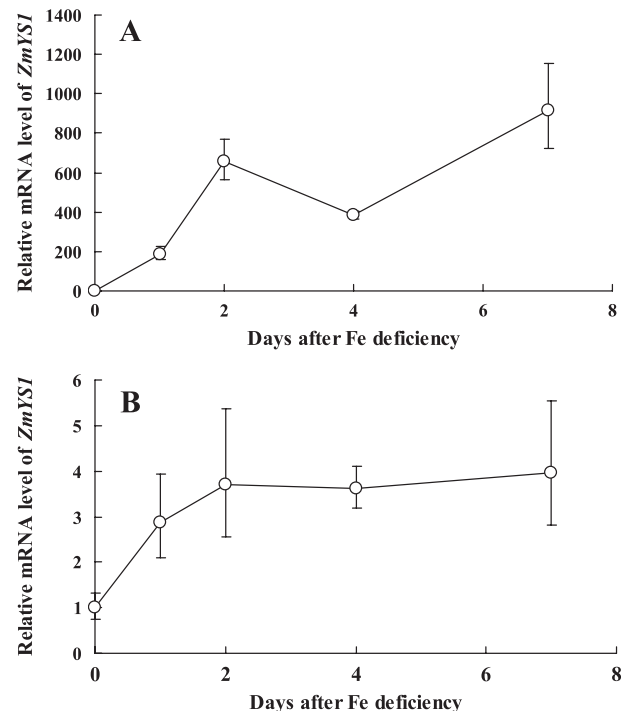
## Results and discussion

### Comparison of the expression pattern of *ZmYS1* and *HvYS1*

Previous studies have shown that the expression of both *ZmYS1* and *HvYS1* was induced by Fe deficiency (Curie *et al.*, 2001; Murata *et al.*, 2006). Northern blot hybridization analysis showed that the expression of *ZmYS1* in maize roots increased with time since the initiation of Fe deprivation (Curie *et al.*, 2001). In the present study, the expression of *HvYS1* was determined at different time points after iron deficiency by using real-time RT-PCR. Similar to *ZmYS1* (Curie *et al.*, 2001), the expression of *HvYS1* increased with the time of Fe deficiency treatment (Fig. 1A). On day 7 after Fe deficiency treatment, the *HvYS1* mRNA abundance in the roots increased 25-fold compared with that on day 0 ( $P < 0.01$ ; Fig. 1A). However, in contrast to *ZmYS1*, the expression levels of *HvYS1* in the shoots were significantly lower than those in the root at each time point ( $P < 0.01$ ; Fig. 1A).

The response of *ZmYS1* to metal deficiency has been investigated in maize. The expression of *ZmYS1* was up-regulated in roots by Fe deficiency, but not by Cu or Zn deficiency (Roberts *et al.*, 2004). A similar experiment was conducted with *HvYS1*. As shown in Fig. 1B, deficiency of Mn, Cu, or Zn for 7 d did not induce the expression of *HvYS1* ( $P > 0.05$ ). These results indicate that like *ZmYS1*, *HvYS1* is not involved in the primary acquisition of Mn, Zn, and Cu.

Since *ZmYS1* is also expressed in the shoots (Curie *et al.*, 2001; Roberts *et al.*, 2004), the expression of *ZmYS1* was



**Fig. 4.** Time-dependent expression of *ZmYS1* in the youngest leaf blades (A) and seminal roots (B). Maize seedlings were grown in a nutrient solution without Fe for different numbers of days. The expression level of *ZmYS1* in each tissue on different days was determined by quantitative RT-PCR. The mRNA expression level as relative mRNA levels was normalized, respectively, based on the expression level of different tissues of Fe-sufficient plants. Data are means  $\pm$  SD ( $n=3$ ).

further examined in different tissues of Fe-deficient maize. The highest abundance of *ZmYS1* was found in the leaf blades (Fig. 2). The expression of *ZmYS1* was much lower in the leaf sheaths, and almost no expression was detected in the hypocotyl. In crown and seminal roots, the expression of *ZmYS1* was similar ( $P > 0.05$ ) and lower than that in the leaf blades ( $P < 0.01$ ; Fig. 2).

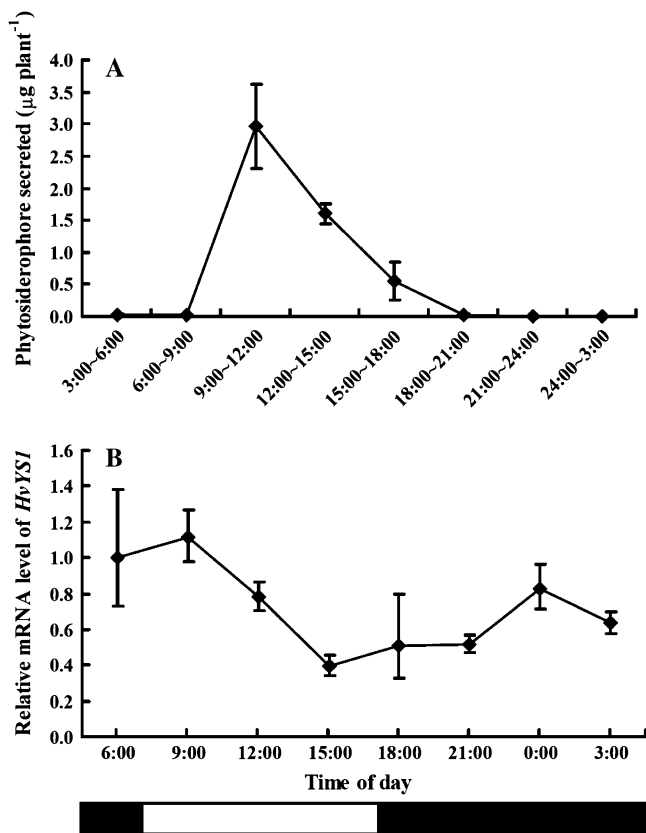
The expression of *ZmYS1* was then further examined in different leaf blades with different Fe status. To generate leaf blades with different Fe status, maize seedlings were first grown in a nutrient solution containing Fe and then transferred to a solution without Fe. After 4 d in the -Fe solution, the younger leaves showed Fe deficiency symptoms of chlorosis, while the older leaves stayed green (Fig. 3A). The SPAD value of the youngest leaf without Fe supply was only half of that in plants with continuous Fe supply ( $P < 0.01$ ; Fig. 3B), whereas the oldest leaf had similar SPAD values in the +Fe- and -Fe-treated plants ( $P > 0.05$ ). Notably, the expression level of *ZmYS1* tended to increase with decreasing SPAD value in leaf blades, with >20-fold difference between the youngest and the oldest leaf blade ( $P < 0.01$ ; Fig. 3C). These results indicate that the

expression of *ZmYS1* is regulated by Fe status in leaf blades.

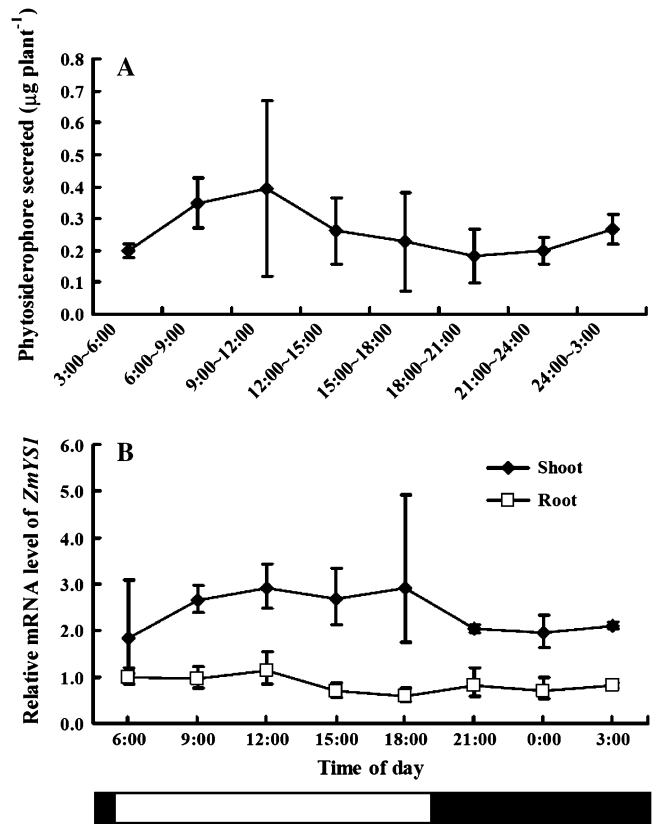
The time-dependent changes of *ZmYS1* expression were further investigated in the youngest leaf blade and roots. The expression of *ZmYS1* increased rapidly from day 1 after Fe deficiency, and reached 600 times higher on day 2 compared with the expression level of the leaf blade from the +Fe plants (Fig. 4A). The expression in the seminal roots was also enhanced, reaching a plateau 1 d after the Fe deficiency treatment ( $P < 0.01$ ), but the expression levels were only 3- to 4-fold higher than those of the Fe-sufficient plants. (Fig. 4B). All these results indicate that the expression of *ZmYS1* responds to the Fe deficiency more in the youngest leaf blade than in the roots, which is different from *HvYS1* (Fig. 1A).

*Diurnal rhythm of ZmYS1 and HvYS1 expression*

Secretion of phytosiderophore from the roots is characterized by a diurnal rhythm in some gramineous plants such as barley, wheat, and *Festuca rubra* (Takagi *et al.*, 1984;



**Fig. 5.** Diurnal rhythm of phytosiderophore secretion (A) and *HvYS1* expression (B) in barley. Root exudates were collected from Fe-deficient barley seedlings every 3 h in a glasshouse (sunrise 7:00 h) and roots were sampled at the same time point. The amount of phytosiderophore secreted was determined according to Ma *et al.* (2003). The expression level of *HvYS1* at different times was determined by quantitative RT-PCR. The relative mRNA levels in the roots at 6:00 h are shown. Data are means  $\pm$ SD ( $n=3$ ).



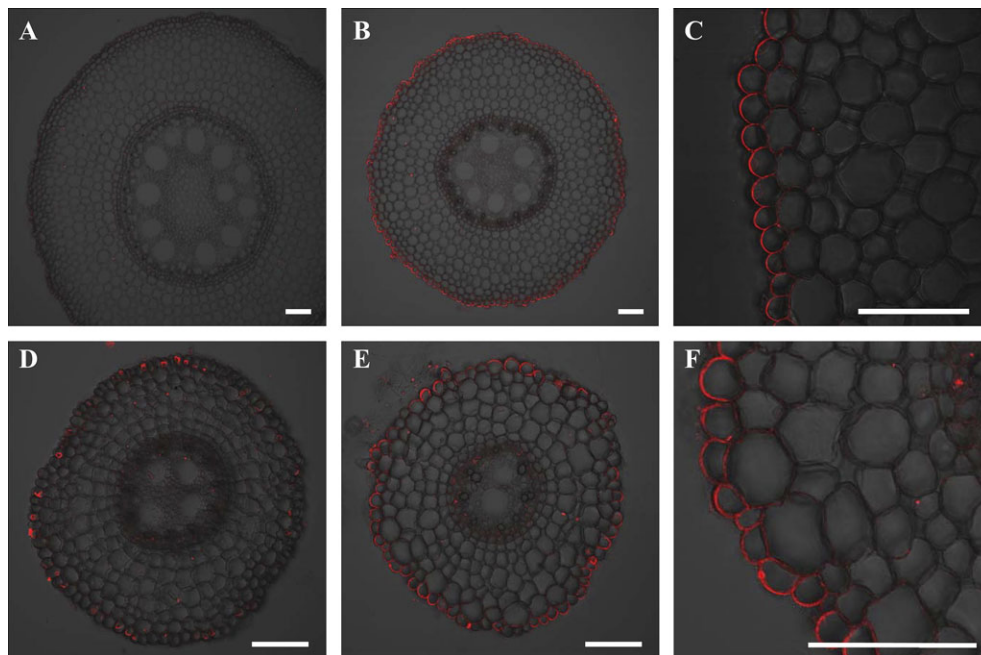
**Fig. 6.** Diurnal rhythm of phytosiderophore secretion (A) and *ZmYS1* expression (B) in maize. Root exudates were collected from Fe-deficient maize seedlings every 3 h (sunrise 5:00 h) and roots and shoots were sampled at the same time point. The amount of phytosiderophore secreted was determined by HPLC according to Ueno *et al.* (2007). The expression level of *ZmYS1* at different times was determined by quantitative RT-PCR. The relative mRNA levels in the roots at 6:00 h are shown. Data are means  $\pm$ SD ( $n=3$ ).

Ma *et al.*, 2003; Reichman and Parker, 2007). Generally, plants start to secrete phytosiderophores in the morning from the third hour after sunrise or the onset of the light period, and secretion continues for ~3 h. However, gramineous plants such as maize do not show a diurnal rhythm in secretion (Yehuda *et al.*, 1996). To investigate whether the expression of *ZmYS1* and *HvYS1* has a diurnal rhythm, the time-dependent expression of *HvYS1* and *ZmYS1* as well as phytosiderophore secretion were determined in Fe-deficient barley and maize. In Fe-deficient barley, the secretion of phytosiderophore showed a diurnal rhythm ( $P < 0.01$ ; Fig. 5A). With sunrise occurring at 7:00 h, the secretion reached a peak during 9:00–to 12:00 h. This result is in agreement with previous findings (Takagi *et al.*, 1984). In contrast to the phytosiderophore secretion pattern, *HvYS1* was expressed all day in the Fe-deficient barley roots (Fig. 5B). However, there was a significant fluctuation in the expression level ( $P < 0.01$ ). The peak of expression was found at 6:00 h, which was before sunrise (Fig. 5B), and remained at a constant level at 9:00 h, during the peak of phytosiderophore secretion. This result is not completely consistent with that of Nagasaka *et al.* (2009). They found that the expression peak of *HvYS1* was at the start of illumination. Since they did not monitor the secretion of phytosiderophore at the same time, it is difficult to compare their results directly with the present results. Phytosiderophore is easily degraded by microorganisms (Watanabe and Wada, 1989); therefore, the expression of *HvYS1* before the secretion of phytosiderophore will help the roots to take up the Fe(III)–phytosiderophore complex efficiently.

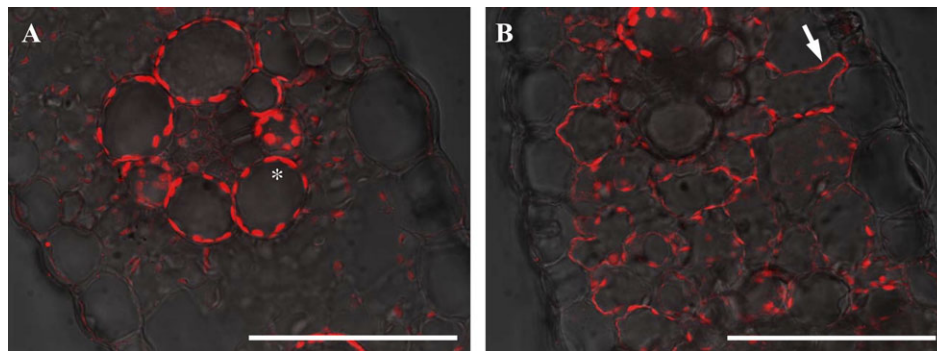
In contrast, the secretion of phytosiderophore in the Fe-deficient maize roots did not show a distinct diurnal rhythm as seen in barley ( $P > 0.05$ ; Fig. 6A). This is in agreement with previous findings (Yehuda *et al.*, 1996). Interestingly, there was also no diurnal change in the expression of *ZmYS1* in either the roots or shoots ( $P > 0.05$ ; Fig. 6B). Although the mechanism controlling the diurnal rhythm of phytosiderophore secretion and *YS1* expression is still unknown, it would be interesting to compare the promoter regions of *HvYS1* and *ZmYS1* in the future.

#### Tissue-type specificity of localization of *ZmYS1*

Immunostaining showed that *HvYS1* is localized to the epidermal cells in the Fe-deficient barley roots (Murata *et al.*, 2006). However, in maize, the tissue-type specificity of localization of *ZmYS1* is unknown. The localization was investigated by immunostaining with anti-*ZmYS1* polyclonal antibody. In the Fe-sufficient maize, the signal of *ZmYS1* was hardly observed in both the crown and lateral roots (Fig. 7A, D). However, in the Fe-deficient maize, *ZmYS1* was found to be localized at the epidermal cells of crown and lateral roots (Fig. 7B, E). This localization is similar to *HvYS1* (Murata *et al.*, 2006). However, different from *HvYS1*, *ZmYS1* showed polar localization at the distal side of the epidermal cells. Polar localization of some mineral transporters has been reported recently. For example, an influx silicon transporter *Lsi1* is localized at the distal side of the exodermal and endodermal cells of rice roots (Ma *et al.*, 2006), while an efflux silicon transporter *Lsi2* is localized at the proximal side of the same cells (Ma *et al.*, 2007). In maize roots, the Si influx transporter



**Fig. 7.** Tissue-specific localization of *ZmYS1* in maize roots. (A, D) Immunostaining with anti-*ZmYS1* antibody in the crown (A) and lateral roots (D) of maize grown in a nutrient solution containing Fe. (B, C, E, and F), Immunostaining in the crown (B and C) and lateral roots (E and F) of maize grown in Fe-free nutrient solution. Scale bars indicate 100  $\mu\text{m}$ .



**Fig. 8.** Localization of ZmYS1 in maize leaf blade. A leaf blade of plants grown in Fe-sufficient (A) or Fe-deficient conditions with chlorosis (B) was used for immunostaining. The asterisk and arrow indicate autofluorescence of the chloroplast and ZmYS1 localization at the plasma membrane of the mesophyll cell, respectively. Scale bars indicate 100  $\mu\text{m}$ .

ZmLsi1 also showed polar localization at the distal side of epidermal and cortical cells (Mitani *et al.*, 2009). To our knowledge, this is the first time that polar localization has been described for a metal complex transporter. The mechanism controlling polar localization of transporters has not been described. It will be interesting to elucidate the mechanism of polar localization by comparing HvYS1 and ZmYS1 in the future.

In the leaf blade, ZmYS1 was not localized in the epidermal cells, but was detected on the peripheries of all mesophyll cells (Fig. 8). Heterologous studies in yeast and *Xenopus* oocytes have shown that ZmYS1 transports not only the Fe(III)-phytosiderophore complex, but also zinc-, copper-, and nickel-phytosiderophore complexes, and nickel, Fe(II), and Fe(III) complexes with NA (Roberts *et al.*, 2004; Schaaf *et al.*, 2004). The localization of ZmYS1 in the leaf blade suggests that ZmYS1 is also involved in the intracellular transport of these metals. However, since the expression of ZmYS1 in both roots and shoots only responded to Fe deficiency, but not to Cu and Zn deficiency at both the mRNA and protein levels (Roberts *et al.*, 2004), it would be interesting in the future to examine whether ZmYS1 is also involved in intracellular transport of Zn and Cu in *planta*.

The present results together with previous findings indicate that HvYS1 is only involved in primary uptake of iron from the rhizosphere to the root epidermal cells in barley, while ZmYS1 has dual roles, both iron acquisition from the rhizosphere and the intracellular transport of iron and possibly other metals in the leaf blades. Since the intracellular transport of iron in barley leaves is also required, it will be interesting to identify Fe transporter(s) in barley leaves in the future. According to the database, there are >10 expressed sequence tags showing homology to *HvYS1*. Functional analysis of these homologues in the future will help understand the iron transport system in whole plants.

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## References

- Curie C, Briat JF. 2003. Iron acquisition and signaling in plants. *Annual Review of Plant Biology* **54**, 183–206.
- Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL. 2001. Maize *yellow stripe 1* encodes a membrane protein directly involved in Fe (III) uptake. *Nature* **409**, 346–349.
- DiDonato RJ, Roberts LA, Sanderson T, Easley RB, Walker EL. 2004. *Arabidopsis Yellow Stripe-Like2 (YSL2)*: a metal-regulated gene encoding a plasma membrane transporter of nicotianamine-metal complexes. *The Plant Journal* **39**, 403–414.
- Gendre D, Czernic P, Conejero G, Pianelli K, Briat JF, Lebrun M, Mari S. 2007. TcYSL3 a member of the YSL gene family from the hyper-accumulator *Thlaspi caerulescens* encodes a nicotianamine Ni/Fe transporter. *The Plant Journal* **49**, 1–15.
- Inoue H, Kobayashi T, Nozoye T, Takahashi M, Kakei Y, Suzuki K, Nakazono M, Nakanishi H, Mori S, Nishizawa NK. 2009. Rice OsYSL15 is an iron-regulated iron(III)-deoxymugineic acid transporter expressed in the roots and is essential for iron uptake in early growth of the seedlings. *Journal of Biological Chemistry* **284**, 3470–3479.
- Jean ML, Schikora A, Mari S, Briat JF, Curie C. 2005. A loss-of-function mutation in AtYSL1 reveals its role in iron and nicotianamine seed loading. *The Plant Journal* **44**, 769–782.
- Koh S, Wiles AM, Sharp JS, Naider FR, Becker JM, Stacey G. 2002. An oligopeptide transporter gene family in *Arabidopsis*. *Plant Physiology* **128**, 21–29.
- Koike S, Inoue H, Mizuno D, Takahashi M, Nakanishi H, Mori S, Nishizawa NK. 2004. OsYSL2 is a rice metal-nicotianamine transporter that is regulated by iron and expressed in the phloem. *The Plant Journal* **39**, 415–424.
- Ma JF. 2005. Plant root responses to three abundant soil mineral: silicon, aluminum and iron. *Critical Reviews in Plant Sciences* **24**, 267–281.

- Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, Ishiguro M, Murata Y, Yano M.** 2006. Silicon transporter in rice. *Nature* **440**, 688–691.
- Ma JF, Ueno H, Ueno D, Rombolà AD, Iwashita T.** 2003. Characterization of phytosiderophore secretion under Fe deficiency stress in *Festuca rubra*. *Plant and Soil* **256**, 131–137.
- Ma JF, Yamaji N, Mitani N, Tamai K, Konishi S, Fujiwara T, Katsuhara M, Yano M.** 2007. An efflux transporter of silicon in rice. *Nature* **448**, 209–211.
- Marschner H, Römheld V.** 1994. Strategies of plants for acquisition of iron. *Plant and Soil* **165**, 261–274.
- Mitani N, Yamaji N, Ma JF.** 2009. Identification of maize silicon influx transporters. *Plant and Cell Physiology* **50**, 5–12.
- Murata Y, Ma JF, Yamaji N, Ueno D, Nomoto K, Iwashita T.** 2006. A specific transporter for iron(III)-phytosiderophore in barley roots. *The Plant Journal* **46**, 563–572.
- Nagasaka S, Takahashi M, Nakanishi-Itai R, Bashir K, Nakanishi H, Mori S, Nishizawa NK.** 2009. Time course analysis of gene expression over 24 hours in Fe-deficient barley roots. *Plant Molecular Biology* **69**, 621–631.
- Reichman SM, Parker DR.** 2007. Probing the effects of light and temperature on diurnal rhythms of phytosiderophore release in wheat. *New Phytologist* **174**, 101–108.
- Roberts LA, Pierson AJ, Panaviene Z, Walker EL.** 2004. Yellow Stripe1. expanded roles for the maize iron-phytosiderophore transporter. *Plant Physiology* **135**, 115–120.
- Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N.** 2004. ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. *Journal of Biological Chemistry* **279**, 9091–9096.
- Schaaf G, Schikora A, Häberle J, Vert GA, Ludewig U, Briat JF, Curie C, von Wirén N.** 2005. A putative function of the Arabidopsis Fe-phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. *Plant and Cell Physiology* **46**, 762–774.
- Takagi S.** 1976. Naturally occurring iron-chelating compounds in oat- and rice-root washing. *Soil Science and Plant Nutrition* **22**, 423–433.
- Takagi S, Nomoto K, Takemoto T.** 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *Journal of Plant Nutrition* **7**, 469–477.
- Ueno D, Rombolá A, Iwashita T, Nomoto K, Ma JF.** 2007. Identification of two novel phytosiderophores secreted from perennial grasses. *New Phytologist* **174**, 304–310.
- Walker EL, Connolly EL.** 2008. Time to pump iron: iron-deficiency-signaling mechanisms of higher plants. *Current Opinion in Plant Biology* **11**, 530–535.
- Watanabe S, Wada H.** 1989. Mugineic acid-decomposing bacteria isolated from rhizosphere of iron-deficient barley. *Japanese Journal of Soil Science and Plant Nutrition* **60**, 413–417.
- Waters BM, Chu HH, DiDonato RJ, Roberts LA, Easley RB, Lahner B, Salt DE, Walker EL.** 2006. Mutations in Arabidopsis *Yellow Stripe-Like1* and *Yellow Stripe-Like3* reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiology* **141**, 1446–1458.
- Yehuda Z, Shenker M, Römheld V, Marschner H, Hadar Y, Chen Y.** 1996. The role of ligand exchange in the uptake of iron from microbial siderophores by gramineous plants. *Plant Physiology* **112**, 1273–1280.
- Yen MR, Tseng YH, Saier MH.** 2001. Maize *Yellow Stripe1*, an iron-phytosiderophore uptake transporter, is a member of the oligopeptide transporter (OPT) family. *Microbiology* **147**, 2881–2883.