Improving Rice Tolerance to Low Iron Availability in Alkaline Soils by Using the Genes Participated in Iron Acquisition Strategies

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Abstract

One of the widest ranging abiotic stresses in world agriculture arises from low iron (Fe) availability due to high soil pH, with 30% of arable land too alkaline for optimal crop production. Rice is very susceptible to low Fe availability because of a low capacity to secrete mugineic acid family phytosiderophores (MAs), which are Fe chelators secreted by graminaceous plants. This is in contrast to barley that has high secretion of MAs and is efficient in Fe uptake. We tested transgenic rice lines possessing three barley genes involved in MAs synthesis in a field experiment on a calcareous soil under paddy conditions. Two rice lines, one with a barley nicotianamine synthase gene and one with a barley *IDS3* gene encoding a dioxygenase showed higher tolerance to low Fe availability. This was evident in better early growth with plants suffering less Fe-deficiency chlorosis. These results show that introducing barley genes involved in the synthesis of MAs into rice is an effective, practical method to improve agricultural productivity in calcareous soils.

Media summary

Improving rice tolerance to low iron availability in calcareous soils by introducing genes in phytosiderophore synthesis is an effective method to increase in agricultural productivity.

Key words

Agricultural productivity, genes in MAs synthesis, transgenic rice

Introduction

Fe availability in calcareous soils is very low due to high soil pH which reduces the solubility of ferric iron. Graminaceous plants have a specific uptake mechanism for precipitated ferric iron in soils; they secrete metal chelators known as mugineic acid family phytosiderophores (MAs) into the rhizosphere (Takagi, 1976) and absorb the Fe(III)-MAs complex from the soil through the Fe(III)-MAs complex transporter YS1 (Curie et al., 2001). MAs are synthesized from methionine (Mori and Nishizawa, 1987; Figure 1).

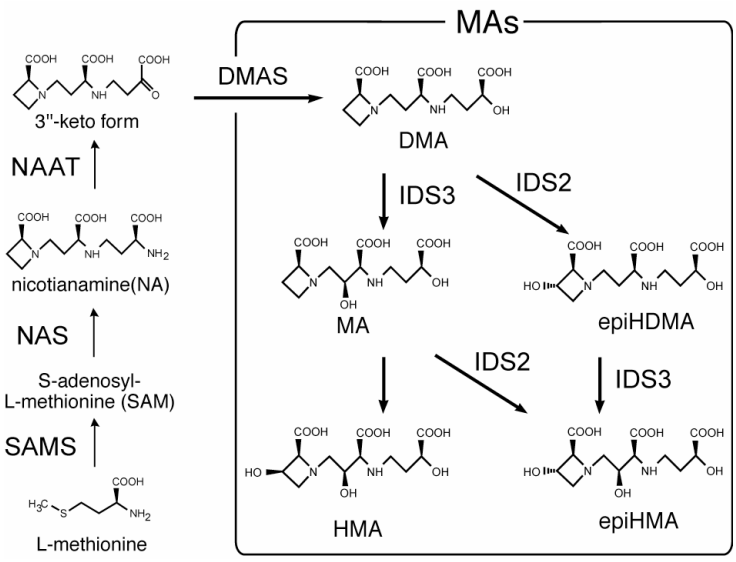

Figure 1. Biosynthesis pathway of mugineic family phytosiderophores (MAs).
SAMS, S-adenosyl-methionine synthetase; NAS, nicotianamine synthase; NAAT, nicotianamine aminotransferase; DMAS, deoxymugineic acid synthase; IDS2 (IDS3), iron deficiency specific clone no. 2 (3); DMA, 2'-deoxymugineic acid; MA, mugineic acid; HMA, hydroxymugineic acid; epiHDMA, epi-hydroxydeoxymugineic acid; epiHMA, epi-hydroxymugineic acid

The genes that encode all enzymes in the biosynthetic pathway of MAs from barley roots have previously been isolated. Rice is much more susceptible to low Fe availability than other graminaceous plants due to low MAs-secretion capacity. We enhanced the tolerance of rice to Fe deficiency in calcareous soils by introducing the barley genes participating in the MAs production. In this work, Takahashi et al. (2001) produced a transgenic rice line possessing the barley *NAAT* gene with enhanced tolerance of rice to low Fe availability through increased DMA; Kobayashi et al. (2001) showed that a transgenic rice line possessing the barley *IDS3* gene secreted both DMA and MA; and Higuchi et al. (2001) produced a transgenic rice line

possessing the barley *HvNAS1* gene with enhanced NAS activity in Fe-deficient roots. We have also produced a transgenic rice line possessing both the *HvNAS1* and *HvNAAT* genes. The focus of the present study was to evaluate the tolerance of these transgenic rice lines to low Fe availability in a calcareous soil under paddy conditions in the field.

Methods

A total of five rice (*Oryza sativa* L. cv. Tsukinohikari) lines, three being transgenic were tested in the field experiment. Here, the rice line possessing the 13.5-kb genome fragment of *HvNAS1* gene (Higuchi et al., 2001a) was defined as "gNAS1"; that possessing the 7.6-kb genome fragment of *HvNAS1* and 11-kb genome fragment of *HvNAAT* genes as "gNAS1-gNAAT", the 11-kb genome fragment of *HvNAAT* being the same as that used by Takahashi et al. (2001); and that possessing the 20-kb genome fragment of *IDS3* gene (Kobayashi et al., 2001) as "gIDS3". Healthy seedlings were transplanted (three per hill) in the calcareous paddy field on 18 May 2006. The water in the paddy was maintained at > 6 cm deep throughout the experiment except two weeks before harvest. The experimental plots were arranged in a completely randomized design, with three replicates of each transgenic line and four replicates of NT.

Results

There was good growth of all lines during the first 16 DAT; thereafter, chlorosis was evident in some lines which also grew less vigorously. By 42 DAT, the three transgenic rice lines were clearly superior to NT (Figure 4a) both in leaf color and growth, though there were difference in performance in individual plots. The clearest difference between gIDS3 and NT was evident 42 DAT, but one week later (50 DAT), leaf chlorosis began to disappear, especially in NT plants located close to the plot boundary between adjacent to the transgenic gIDS3 rice plants. The visible assessment of leaf color was confirmed by the measured SPAD values. While there was no significant difference ($P<0.05$) in plant height among rice lines at 16 DAT, gNAS1, gNAS1cal, and gIDS3 plants were higher than those of NT from 25 DAT. These plants continued to increase in height (5.6 - 2.8 cm) until 42 DAT, unlike NT plants which did not. The number of tillers per plant 16 and 25 DAT differed among the lines tested, with that of gIDS3 being significantly higher than that of other transgenic rice lines and NT. The heading stage occurred around 100 DAT (25 August), and the plants were harvested on 152 DAT (23 October).

The number of grains in gNAS1 was significantly higher ($P < 0.05$) than those of NT and the number of grains tended to be higher in transgenic rice lines than in NT (Table 1). The 1000-grain weights of both gNAS1 and gNAS1cal were higher than that of NT, while that of gNAS1-gNAAT and gIDS3 were almost the same as that of NT. The grain yield of gNAS1 and gNAS1cal tended to be higher than that of NT, but not significantly so ($P < 0.05$).

Conclusion

Transgenic rice lines possessing three barley genes involved in MA synthesis that improve Fe nutrition clearly grew better than NT after transplanting in the calcareous paddy field. This was especially evident during early growth. In conclusion, we have shown that a transgenic approach to increase the tolerance of rice to low Fe availability is a practical way to improve agricultural productivity in calcareous paddy soils. This strategy may also apply to other graminaceous crops such as upland rice, maize and sorghum that have reduced amounts of MAs secretion. Further opportunities for improving Fe nutrition of crops involve the recently-reported novel bHLH transcription factor induced by Fe deficiency (IRO2) in rice and barley (Ogo et al., 2007) and the synthetic ferric chelate reductase gene (*refre1-372*) in rice that has been selected for improved activity at high pH (Ishimaru et al., 2007).

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