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Data in Brief

Transcriptomic analyses of maize ys1 and ys3 mutants reveal maize iron homeostasis

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ABSTRACT

To acquire iron (Fe), graminaceous plants secrete mugineic acid family phytosiderophores (MAs) (Takagi, 1976 [1]) through the MAs efflux transporter TOM1 (Nozoye et al., 2011 [2]) and take up Fe in the form of Fe(III)–MAs complexes through the Fe(III)-MAs transporter YS1 (Curie et al., 2001 [3]). Yellow stripe 1 (ys1) and ys3 are recessive mutants of maize (*Zea mays* L.) that result in symptoms typical of Fe deficiency, i.e., interveinal chlorosis of the leaves. The ys1 mutant is defective in the YS1 transporter and is therefore unable to take up Fe(III)–MAs complexes. While the ys3 mutant has been shown to be defective in MA release, the causative gene has not been identified. The objective of the present work was to identify the genes responsible for the ys1 and ys3 phenotypes, so as to extend our understanding of Fe homeostasis in maize by qRT-PCR. In agreement with previous reports, the expression level of YS1 was decreased in the ys1 mutant. Moreover, we identified that the expression level of a homolog of TOM1 in maize (ZmTOM1) was significantly decreased in the ys3 mutant. Here described the quality control and analysis that were performed on the dataset. The data is publicly available through the GEO database with accession number GSE44557. The interpretation and description of these data are included in a manuscript (Nozoye et al., 2013 [4]).

Specifications	
Organism/cell line/tissue	*Zea mays*
Sex	N/A
Sequencer or array type	StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA)
Data format	Normalized with housekeeping genes: ZmUbiquitin; GRMZM2G118637.
Experimental factors	ys1 mutant vs. WT, ys3 mutant vs. WT under Fe-sufficient or Fe-deficient condition
Experimental features	qRT-PCR gene expression profiling to identify the genes responsible for the ys1 and ys3 phenotypes.
Consent	N/A
Sample source location	The University of Tokyo, Tokyo, Japan

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44557.

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2. Experimental design, materials and methods

2.1. Plant materials

The homozygous seeds of *Yellow stripe 1* (*ys1*) and *ys3* mutant plants were used to identify the genes responsible for the *ys1* and *ys3* phenotypes, and to extend our understanding of Fe homeostasis in maize. A WT cultivar (Alice) was used as a control, as even though it has a different genetic background from *ys1* [3] and *ys3* [4], this line was previously used in a study of the *ys1* mutant [5].

Seedlings germinated for 4 days in the dark at 25 °C were grown hydroponically in a nutrient solution that contained 0.7 mM K_2SO_4, 0.1 mM KCl, 0.1 mM KH_2PO_4, 2.0 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 10 mM H_3BO_3, 0.5 mM $MnSO_4$, 0.2 mM $CuSO_4$, 0.5 mM $ZnSO_4$, 0.05 mM Na_2MoO_4, and 0.1 mM Fe(III)-EDTA. Fe deficiency was initiated 8 days after germination by transfer of the plants to Fe(III)-EDTA-free culture medium. Maize plants grown hydroponically under Fe-sufficient or Fe-deficient conditions for 5 days were harvested at the same time.

2.2. RNA preparation

The maize plants grown hydroponically were immediately frozen in liquid nitrogen. Total RNA was extracted from the shoots and roots of

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three plants per treatment using an RNeasy Plant Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The yield and purity of the RNA were determined spectrophotometrically. To confirm the biological replicates, RNA was separately extracted from the shoots and roots of three to five plants per treatment.

2.3. Quantitative real-time PCR

Total RNA (3 μg) was treated with RNase-free DNase I (Takara, Kyoto, Japan) to remove contaminating genomic DNA. First-strand cDNA was synthesized using ReverTra Ace reverse transcriptase (Toyobo, Tokyo, Japan) by priming with oligo-d$(T)_{30}$. For quantitative RT-PCR, a fragment was amplified by PCR in a StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA) with SYBR Green I and ExTaq™ Real-Time PCR Version (Takara) according to the manufacturers' instructions. The template concentration was adjusted to 30 ng per reaction. The primers used for real-time PCR are described in Table 1. The primers used as the internal control (*ZmUbiquitin*, GRMZM2G118637) in RT-PCR were as follows: *ZmUbiquitin* forward, 5′-GTTGAAGCTGCTGCTGTA TCTGG-3′ and *ZmUbiquitin* reverse, 5′-GCGGTCGCACGATAGTTTTG-3′.

2.4. Data normalization

Because the maize genes analyzed in this study were not cloned, normalization of quantitative real-time PCR was performed by the comparative Ct method calculation according to the manufacturer's instructions (Applied Biosystems StepOnePlus™ Real-Time PCR system). Briefly, relative quantitation was calculated as follows. The threshold cycle (Ct) indicates the fractional cycle number at which the quantity of amplified target reaches a specified threshold. First, the amount of target was normalized to endogenous reference. There is a relation between the amount of the endogenous reference and the amount of PCR products by Ct value as follows: the amounts of PCR products by Ct value = the amount of the endogenous reference X 2Ct. Therefore, △Ct value is calculated as follows: △Ct = Ct value of the target – Ct value of the endogenous reference. Secondly, the difference between the calibrator which is used as base and the △Ct is calculated as follows: △△Ct = △Ct value of the target - △Ct value of the endogenous reference. Finally, the relative quantitative value is calculated as follows: the relative quantitative value = ^{2}-△△Ct. Since the relative quantitative value of the calibrator is 20 = 1, the other samples are calculated as the relative quantitative value when the value of calibrator is 1.The principle of the comparative Ct method calculation is described in ABI PRISM® 7700 Sequence Detection Systems User Bulletin #2 (www.appliedbiosystems.co.jp).

Fig. 1. Agarose gel electrophoresis of the amplified fragments by RT-PCR.

Table 1
Primers used for quantitative real-time PCR.

Gene name	Gene ID in Gramene	FW (3′–5′)	RV (3′–5′)
ZmMTN	GRMZM2G171111	GCTAGGCTACGAACAATACA	ACATCTGGATTAGGAGTAGG
ZmAPT	GRMZM2G093347	ACAAACTTGGGGACAGGCCA	ACTCTAACTCTCTGAGAAGA
ZmMTK	GRMZM2G464137	CCTGCCTGATATATACAACA	CTTGCTGGCATCCTTGATTG
ZmIDI2	GRMZM2G139533	CTCTTCATTCGGGGGGAGTT	GGAGAGATCAATGGAAGTTA
ZmFDH	GRMZM2G049811	CTGATTCTGCTCAGGAACTT	TCTCCGTAAGAGGTGTGTTG
ZmIDI4	GRMZM2G067265	CTGAGGCAATAGCTGCAACC	AGACCTCATCTGAGAACAGC
ZmRPI	GRMZM2G035599	GGCGTCGTCGAGCACGGCAT	CTTCCTTTCCATGACTGCGA
ZmPRPPs	GRMZM2G065030	GACCTCCGTTTGAGGTTCTG	CGAGCATCGACAGTAAGTAC
ZmIRO2	GRMZM2G057413	AACGACCTCTACTCCTCGCT	CTGCAGCTCCGGGATGTACT
ZmIRO3	GRMZM2G350312	AGATTCATAAGGCTGAGAGG	TCGAAGAGAGTCTACTTGAA
ZmNAS1	GRMZM2G034956	ATCCTGAGGACATTCGTCGC	CAGCGATTTGTAACTATTAT
ZmNAS3	GRMZM2G478568	CGTGTCTACACCACATGCGT	GAGCTAAGCTACATGCTAA
ZmDMAS1	GRMZM2G060952	CTGATCGTGAAGAGCTTCGA	GCAGGGCAGTGGCACGCATT
YS1	GRMZM2G156599	CACTCATCAGCAAGCAGAAA	TGCAGACTCTTAACAGTGAC
ZmTOM1	GRMAM2G063306	AAGTGTAAATTCATGCCGTG	GATCCCCTGGAAAGAAGGCA
ZmTOM2	GRMZM5G877788	GTTTCGTCGGCGCTATCCAT	AAGAACGCGGCATGCTGGCG
ZmTOM3	GRMZM2G141081	GTGCTTTCAGTCACAGGCGT	ATAGGTCCAAGGATTTTGTT
ZmIRT1	GRMZM2G118821	CTCAGATACGAATACGATCA	TCCAGCTCCTCTGCTTCCTC
ZmNRAMP1	GRMZM2G178190	GAGCAAATCATGGGGTCGTT	TGGATCTACAGCACTGTTCC
ZmMATE2/ZmPEZ1	GRMZM2G170128	CTCCTAGGCTTCTACTTTGA	AGTTGGTCCTGAATGTAACC

2.5. Data validation

The data show the relative increase or decrease of the gene expression level in each sample compared to the gene expression levels in Fe-sufficient shoots of the non-transformant (NT) in three experimental replicates and three to five biological replicates. Because the cultivar among *ys1*, *ys3* and WT were different from each other, it was possible that there are polymorphisms in the analyzed genes and the efficiency of PCR are different from each other. Therefore, the sizes and sequences of the amplified fragments were confirmed by agarose gel electrophoresis (Fig. 1) and with an automated sequencer (3130 Genetic Analyzer; Applied Biosystems), respectively. Analysis of variance with the Tukey–Kramer HSD test was used to compare data.

3. Discussion

Herein we described transcriptional profiling of Fe recessive mutants *ys1* and *ys3* in the different cultivars. In this dataset, we confirmed the decrease of YS1 expression level in *ys1* compared to WT as described previously. The expression level of *YS1* was not altered in *ys3*. In addition, we found that the expression level of *ZmTOM1* was decreased in *ys3* compared to WT, but not in *ys1*. Both YS1 [3] and TOM1 [2] are important transporter of MAs [1] which is involved in not only Fe acquisition from soil but also Fe homeostasis in the plant body. We believe that this dataset could provide insights into the characteristics of the YS1 and TOM1 transporters which involved in MAs transport to maintain Fe homeostasis in maize. We believed that the method in this dataset may assist in elucidation of the various mutants which are from different cultivars.

Disclosures

All authors possess no conflicts of interests.

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