OsTH1, a gene involved in vitamin B1 biosynthesis in rice (*Oryza sativa* L.)

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Background

Thiamin deficiency leads to several clinical conditions from mild neurological and psychiatric symptoms to severe encephalopathy, ataxia, congestive heart failure and muscle atrophy[1]. In developing countries, thiamin deficiency remains widespread particularly due to **high rates of white rice consumption**[1]. Rice is thiamine-rich at bran and germ level (removed during polishing). **Biofortification of rice grains by improving the availability of thiamin in rice endosperm** is a one-time investment to combat vitamin B1 deficiency, nonetheless it requires a throughout knowledge of the biosynthetic pathway. Nowadays, most genes involved in thiamin biosynthesis have already been identified in *Arabidopsis* and maize[2]. In rice **only the genes coding for THI1, THIC[3] and TH2[4] were identified**. Here we report the **identification and characterization of a putative *OsTH1* gene** (HMP-P kinase/thiamin monophosphatase synthase), involved in the biosynthesis of thiamin in *Oryza sativa*, opening new doors for rice biofortification.

THIC is the rate-limiting enzyme of B1 biosynthesis in rice while TH1 influences the flux of the pathway

Aiming to identify rate-limiting enzymes of the pathway, we developed a stoichiometric model of vitamin B1 biosynthesis in *Oryza sativa*.

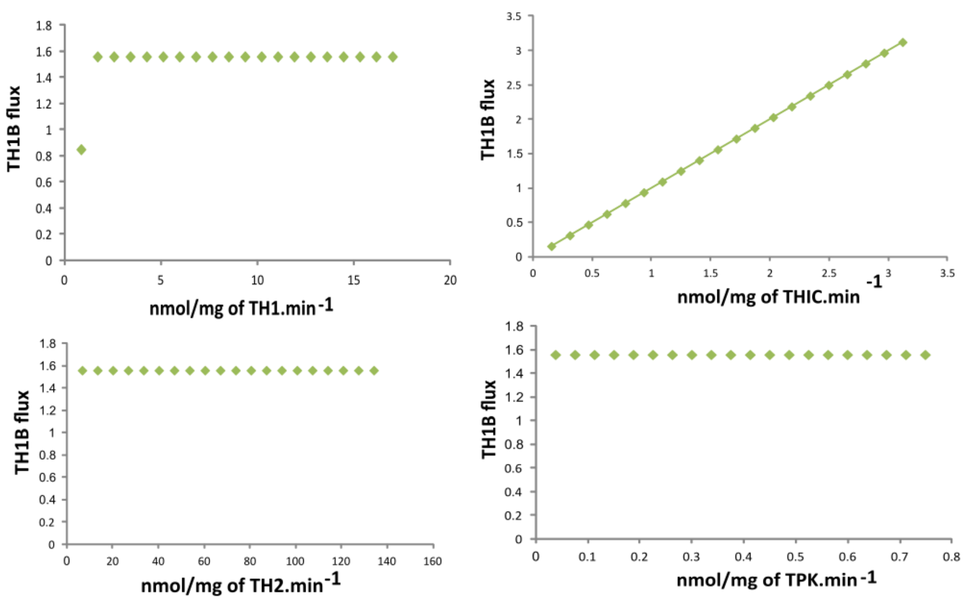


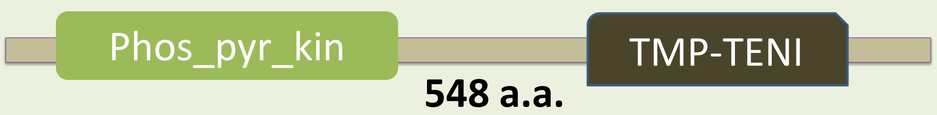
Figure 1. Solution to the FBA optimization problem maximization of TH1B flux.

By running Flux Balance Analysis (FBA) we verified that the **rate-limiting enzyme of the pathway is THIC,** since its activity correlated with the increase flux in TH1B.

By setting the activity of TH1 to 10% of its normal flux, a **decrease in the flux of TH1B was verified**.

LOC_Os12g09000/OsTH1 has all the conserved features of known thiamin monophosphate synthase/HMP-P kinase proteins

Through Blast, we identified **LOC_Os12g09000 as *OsTH1*.**



It contains two conserved domains: **HMP-P kinase domain** and **thiamin monophosphate synthase.**

Additionally, *LOC_Os12g09000* **contains a putative N-terminal chloroplast transit peptide** similarly to other members of this plant protein family.

Aiming to compare the gene expression of the putative LOC_Os12g09000/*OsTH1* with the known *Arabidopsis TH1*, we use GeneVestigator to gather this data. ***OsTH1* has the same expression profile along the different plant organs as *Arabidopsis TH1*.**

Phylogenetic analysis revealed that *OsTH1* clusters with TH1 proteins from Poaceae

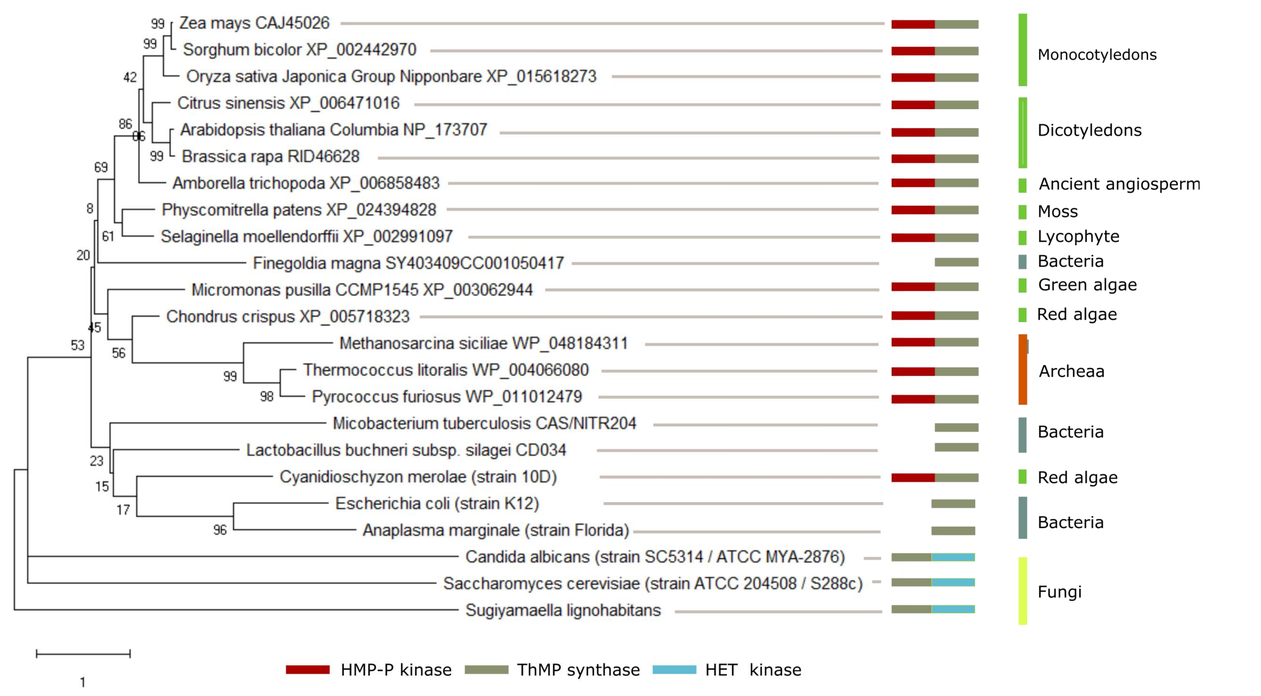


Figure 2. Phylogenetic analysis and domain composition of TH1 proteins .

The putative *OsTH1* is closely related to the bifunctional enzymes from plants, **supporting its putative function**.

OsTH1 functionally complements yeast KO mutants

Due to the genetic tractability and evolutionary conserved cellular pathways of yeasts, we decided to express *OsTH1* in yeast knockout mutants for thiamin monophosphate synthase and HMP-P kinase aiming to functionally validate *LOC_Os12g09000/OsTH1.*

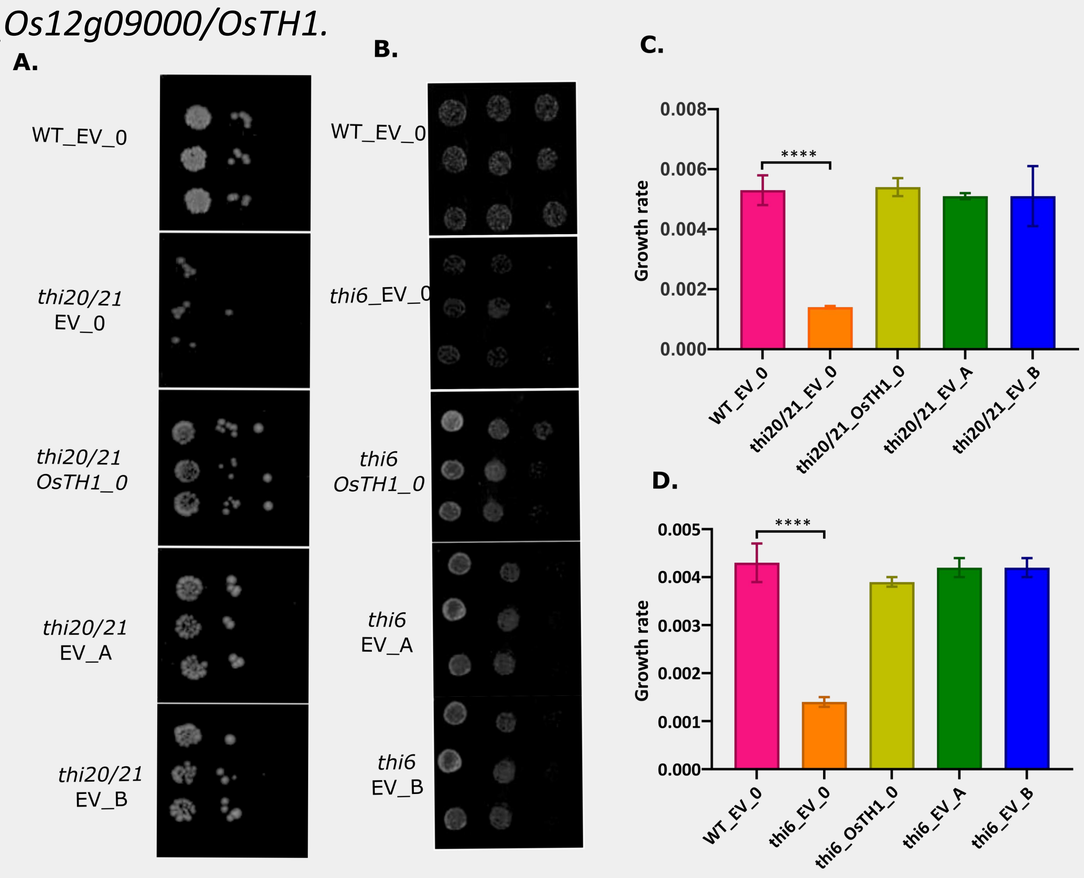


Figure 3. Functional complementation of *thi6Δ* and *thi20/21Δ* yeast mutants. The mutants transformed with the empty vector (EV) were used as negative control with no addition of thiamin exogenously (0), while the wild type transformed with the EV and with no addition of thiamin was use as positive control for all experiments. O, represents 0µM of thiamin, A 1µM and B 2µM **A.** Cell spotting for *thi20/21Δ*, deficient in HMP-kinase. **B.** Cell spotting for *thi6Δ*, deficient in ThMP synthase. **C.** Growth rate of *thi20/21Δ*, deficient in HMP-kinase. **D.** Growth rate of *thi6Δ*, deficient in HMP-kinase.

***OsTH1* expression restored the growth impairment of both *thi6Δ* and *thi20/21Δ* mutants**. Moreover, when adding different concentrations (1µM and 2µM) of thiamin to the medium the growth was also restored.

In the future, **the transformation of rice *calli* with *OsTH1* followed by UHPLC-MS analysis** of the vitamin and precursors and mRNA analysis will be performed. In addition, ***in vitro* activity assays** will also be undertaken in order to fully prove *OsTH1* function.

References: 1.Strobbe, S. & Van Der Straeten, D. Toward eradication of B-vitamin deficiencies: Considerations for crop biofortification. *Frontiers in Plant Science* **9**, 443 (2018).
2.Hsieh, W.Y. *et al.* The Arabidopsis thiamin-deficient mutant pale green1 lacks thiamin monophosphate phosphatase of the vitamin B1 biosynthesis pathway. *Plant J* **91**, 145–157 (2017).
3.Dong, W., Thomas, N., Ronald, P. C. & Goyer, A. Overexpression of thiamin biosynthesis genes in rice increases leaf and unpolished grain thiamin content but not resistance to *Xanthomonas oryzae* pv. *oryzae*. *Front Plant Sci* **7**, 616 (2016).
4.Hsieh, P.-H. *et al.* The rice PALE1 homolog is involved in the biosynthesis of vitamin B1. *Plant Biotechnology Journal*,(2020).

Agnowlegements

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