Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Figure S5

Content:

A. Primers used for cloning of *NtMTP2* promoter

B. *In situ* analysis of the promoter sequences including 5'UTR of *NtMTP2*

A. Primers used for cloning of *NtMTP2* promoter

NtMTP2-prom-for	**CAC CTG TTA GTG TTT AGG ATA GAG TAG**
NtMTP2-prom-rev	**GTT TAG AAG TTC TAT GAA ATT TGG GAG A**

CACC – sequence added to clone the insert into the pENTR plasmid in proper orientation.

Cloned promoter sequence of *NtMTP2* + 5'UTR + START codon (contig AWOK01S500825.1 position 3015 …4286 – without ATG),

In red - START codon.
Underlined - sequence of the forward primer and the sequence complementary to the reverse primer.

TGTTAGTGTTTAGGATAGAGTAGGATATTTTCAAGAGGGGATTTGCAACGTGAGATGGTTGTAAACCTGTAATGTCAGCAT
GTTACATCACTTTATTCTGTTTCCCAATCGATACCTTTAGATTACATGTCAACAGCAAAAAGAAGAAAAAAAATGAAATTT
AGCTCACTTGCAGTCATCCTAGTACTTTTGGTAGAAGTGAAGATTATTTGCTAATTGAGTGTTTTTTTTCGTGCAAAGGAT
ATTGTGGAATCGGTCGATAATAAGTGACTTTTTGATCTCTTTATTTTGGTCCAAAATAATTGTCCTTTTATATAATCAAGA
AAAAATTAATTTTTTTTTTTAAAATTCGTCCTTATTTATATATTCAATTGTGTCAAGTTAATAATATGTAGATTTTAATTA
AGGGTAATTTTCAAAATATTTTTTTTCTATAAGAGTATTTTTTTTTAAGGGTGTGCCAAATACAAAAATATTATTTATTAT
GAATGGGAGGGAGTATAAATTAGTCCTGGTCAGGGGTTAACACTATGCCTTGATTGAGTGGACTACTTTTGATCGCTTGTT
GTACAAAACTTTCTATCTTTTGGACCAGCGGTTAGAAAAGATAGTAATTGTTTTACATCAAATGCTATATATCGGACTATA
TGAAATTCAAAATAATTATTTCACCCGTTTCAATTTATGTGACGTGTTAAAAAGAATGATCATTTTTTATAATTTTTATGT
AATGATTTATAATCACACAAAATATATATGTCTTATTTTACCTCACAAGTTTAAAAGAATTTTTTTTTTAAAATTTTGTGT
CCAGTCAAATATATTCGACTGAAATGAAATGGAGGGCAGTAGTTATTATACTACTATTAGTACGTAGTTGTCATTGGCAAA
AGAAAACGAAGCAATATTTGTCGTTCATGATCGGGGTTAGACTTAATAGCTTGGAAATTTTAGGACAATTTAAGTTACACT
TATTAAAATTTAGCTGCAGCTTCGTGATTTTTCTTTTACATTACAAAACATGTAATTTTACATACAAACTACAAAAACAAG
AAAATCAAAAAAGATTGGTGATTATACAACTTGACGGGTACTACGCGAAGTATCTGATTCTTTCTTGCACATCCAAACGAT
TAATTTTTAATGGCGGAAAACGGTAATTAACTATTACAACTCCATGGATAATCACTTAATCAGTTGCTTTCTTTAATCACT
CTTCATCGGCGTCTCAAATCCGAGCAATTCTCCCAAATTTCATAGAACTTCTAAACATG

B. *In situ* analysis of the promoter sequence including 5'UTR of *NtMTP2*

The program PlantCARE: http://bioinformatics.psb.ugent.be/webtools/plantcare/html/ , was used to identify *cis*-acting elements within the promoter region. For analysis the 1271 bp fragment of the promoter region (counting from the START codon upstream) was used.

Table 1. List of cis-acting elements found in promotor MTP2; all elements were marked by colours on the promotor sequence below

Cis-acting regulatory elements	**Sequence**	**Position in *NtMTP2* promotor sequence** (bp upstream ATG)	**Function**	**References**
Metals responsive sequences				
MRE1	TGCACAT	1118	heavy **m**etal-**r**esponsive **e**lements	Zhang and Liu, 2017
MRE2	ATTTAGC TGC	979		
IDE2	CAAGTTT/ A	377, 774	sequence the iron deficiency-responsive element	Ogo et al. 2008
Light responsive sequences				
BOX4	ATTAAT	328	part of a conserved DNA module involved in light responsiveness from *Petroselinum crispum*	Weisshaar at al. 1991; Hiratsuka K et al. 1997.
GT1-motif	GGTTAA	520	light responsive element from *Arabidopsis thaliana*	Hagen et al. 1992
Phytohormone responsive sequences				
ABRE	ACGTG	47, 689	cis-acting element involved in the abscisic acid responsiveness from *Arabidopsis thaliana,*	Shen and Ho, 1995
TGACG-element	AACGAC	687, 1084	cis-acting regulatory element involved in the MeJA-responsiveness from *Hordeum vulgare*	Rouster et al. 1997
Regulation of plant development				
MYB-binding sites	CAACAG	130	MYB binding sites correlated with trichome-specific expression	Fasani et al. 2017
General regulatory elements				
TATA-box	ATATAA	multi-position	core promoter element around -30 of transcription start site	Basehoar et al. 2004
CAAT-box	CAAAT	multi-position	common cis-acting element in promoter and enhancer regions from *Pisum sativum*	Frangeul et al.2004

>MTP2prom

+ GTTAGTGTTT AGGATAGAGT AGGATATTTT CAAGAGGGGA TTTGCAACGT GAGATGGTTG TAAACCTGTA

+ AGAGCAT GTTACATCAC TTTATTCTGT TTCCCAATCG ATACCTTTAG ATTACATGTC AACAGCAAAA

+ AGAAGAAAAA AAATGAAATT TAGCTCACTT GCAGTCATCC TAGTACTTTT GGTAGAAGTG AAGATTATTT

+ GCTAATTGAG TGTTTTTTTT CGTGCAAAGG ATATTGTGGA ATCGGTCGAT AATAAGTGAC TTTTTGATCT

+ CTTTATTTTG GTCCAAAATA ATTGTCCTTT TATATAATCA AGAAAAAATT AATTTTTTTT TTTAAAATTC

IDE2

+ GTCCTTATTT ATATATTCAA TTGTGTCAAG TTAATAATAT GTAGATTTTA ATTAAGGGTA ATTTTCAAAA

+ TATTTTTTTT CTATAAGAGT ATTTTTTTTT AAGGGTGTGC CAAATACAAA AATATTATTT ATTATGAATG

+ GGAGGGAGTA TAAATTAGTC CTGGTCAGGG GTTAACACTA TGCCTTGATT GAGTGGACTA CTTTTGATCG

+ CTTGTTGTAC AAAACTTTCT ATCTTTTGGA CCAGCGGTTA GAAAAGATAG TAATTGTTTT ACATCAAATG

+ CTATATATCG GACTATATGA AATTCAAAAT AATTATTTCA CCCGTTTCAA TTTATGTGAC GTGTTAAAAA

+ GAATGATCAT TTTTTATAAT TTTTATGTAA TGATTTATAA TCACACAAAA TATATATGTC TTATTTTACC

IDE2

+ TCACAAGTTT AAAAGAATTT TTTTTTTAAA ATTTTGTGTC CAGTCAAATA TATTCGACTG AAATGAAATG

+ GAGGGCAGTA GTTATTATAC TACTATTAGT ACGTAGTTGT CATTGGCAAA AGAAAACGAA GCAATATTTG

+ TCGTTCATGA TCGGGGTTAG ACTTAATAGC TTGGAAATTT TAGGACAATT TAAGTTACAC TTATTAAAAT

MRE2

+ TTAGCTGCAG CTTCGTGATT TTTCTTTTAC ATTACAAAAC ATGTAATTTT ACATACAAAC TACAAAAACA

+ AGAAAATCAA AAAAGATTGG TGATTATACA ACTTGACGGG TACTACGCGA AGTATCTGAT TCTTTCTTGC

MRE1

+ ACATCCAAAC GATTAATTTT TAATGGCGGA AAACGGTAAT TAACTATTAC AACTCCATGG ATAATCACTT

+ AATCAGTTGC TTTCTTTAAT CACTCTTCAT CGGCGTCTCA AATCCGAGCA ATTCTCCCAA ATTTCATAGA

+ ACTTCTAAA

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