

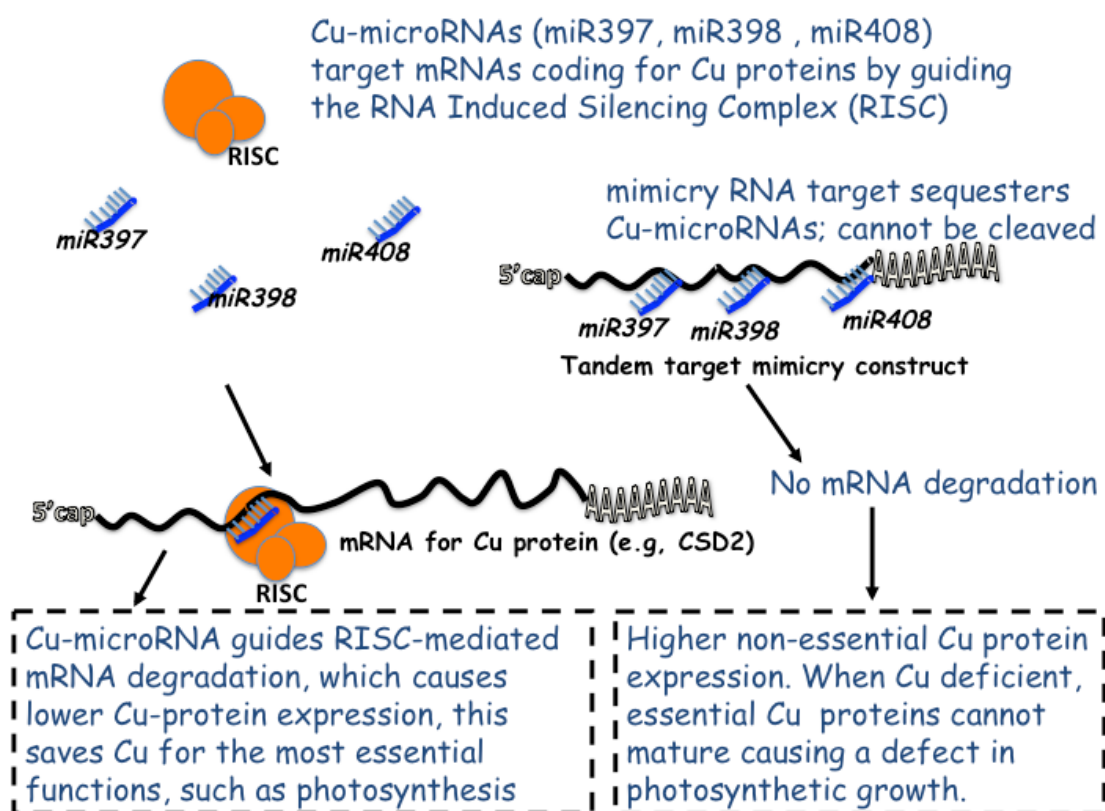
Article

Conserved Cu-MicroRNAs in *Arabidopsis thaliana* Function in Copper Economy under Deficiency

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Supplementary Materials.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: Conceptual model for tandem target mimicry, Table S1: Mineral composition of lines under 3 Cu regimes in mg/kg dw., Table S2: List of the primers used for qRT-PCR and mature miRNA stem-loop qRT-PCR.

Figure S1. Conceptual model for tandem target mimicry and construct sequence**Tandem mimicry-construct sequence**

gcggccgc ccatgg tctaga aaaacaccac aaaaacaaaa gaaaaatggc catcccctag ctaggtgaag
aagaatgaaa acctctaatt tatatagagg ttattcatct tttagcagta atgctaactac actcaatgga
actctctaatt taagtgtttt tgtgttcacg taaggaaagc gttttaagat atggagcaat gaagactgca
gaaggctgat tcagactgcg agttttgttt atctccctct agtaa taggg gtgcctctat gagaacacat
gcttcggttc cctcggaaat cagcagatta tgtatcttta attttgtaat actctctctc ttctctatgc
tttgtttttc ttcattatgt ttgggttgta cccactcccg cgcgt gtcag ggacggctag gcagtgcata
aaaaatattc ggatttgaga actaaaacta gagtagtttt attgatattc ttgtttttca tttagtatct
aataagtttg gagaatagtc agaccggact ttgactctac gaatgtggat ctctttatag tgcattctc
tt gagctc ggatcc gcggccgc

The modified target sequences for respectively miR397, miR398 and miR408 are highlighted. The construct is flanked at the 5' end by *Not1*, *Nco1* and *Xba1* sites and at the 3' end by *Sac1*, *BamH1* and *Not1* sites. The *Xba1* and *Sac1* sites were used for insertion into vector pGWB41.

Table S1 Mineral composition of lines under 3 Cu regimes in mg/kg dw

	Col.			M5			M7			M17		
CuSO₄ Conc.	0 nM	5 nM	50 nM	0 nM	5 nM	50 nM	0 nM	5 nM	50 nM	0 nM	5 nM	50 nM
Ca	3710	3813	3827	3620	4036	3901	4011	4131	4941	4015	3938	4104
	4	6	8	3	7	0	2	2	7	6	3	4
	±	±	±	±	±	±	±	±	±	±	±	±
	1237	1999	1468	1429	544	1359	84	1155	1015	3924	317	61
Fe	42.1	38.3	37.6	43.5	40.1	38.7	43.7	44.9	45.9	45.4	38.9	43.2
	±	±	±	±	±	±	±	±	±	±	±	±
	0.8	3.6	2.3	1.9	0.2	0.4	3.1	3.1	1.9	1.2	1.4	2.5
Mg	3799	3337	3416	3911	3554	3622	4351	3729	3895	4183	3426	3625
	±	±	±	±	±	±	±	±	±	±	±	±
	4.5	223	164	188	30	68	93	57	4.9	10	47	22
Mn	33.2	42.8	37.6	28.3	42.8	38.4	33.1	46.5	42.4	31.2	44.6	40.4
	±	±	±	±	±	±	±	±	±	±	±	±
	0.2	1.8	0.5	2.2	1.3	2.6	0.06	0.6	0.4	1.4	0.7	1.8
Mo	4.3	3.4	2.8	4.6	3.2	2.9	4.2	3.0	2.7	4.2	3.5	3.3
	±	±	±	±	±	±	±	±	±	±	±	±
	0.6	0.2	0.1	0.1	0.5	0.1	0.7	0.2	0.5	0.4	0.1	0.1
P	9739	9639	9437	8306	1052	9904	9875	1154	1062	9864	1035	9672
	±	±	±	±	6	±	±	6	9	±	7	±
	174	805	699	269	±	213	584	±	±	764	±	11
					48			144	791		32	
K	2721	3083	2976	2520	3202	2990	2848	3415	2951	2855	3260	3058
	1	2	1	9	7	0	0	3	0	9	6	6
	±	±	±	±	±	±	±	±	±	±	±	±
	655	2111	293	392	928	239	1278	1287	1542	230	48	15
S	8846	8235	7880	7691	8758	8175	8277	8411	7675	9696	8863	8271
	±	±	±	±	±	±	±	±	±	±	±	±
	458	873	931	279	452	327	327	1134	517	1678	428	104
Zn	42.1	28.1	34.8	37.9	29.1	35.1	46.3	27.3	37.3	38.8	30.1	36.4
	±	±	±	±	±	±	±	±	±	±	±	±
	2.6	2.5	1.1	1.8	0.5	2.7	1.5	1.2	0.9	2.7	0.1	0.2

Table S2, List of the primers used qRT-PCR and mature miRNA stem-loop qRT-PCR**qRT-PCR primers**

	Sequence (5' to 3')	Sense
CSD1	GCCTGGCTACTGGAAACGC	Forward
	GCTTAGTGTGGCTCAAAGCAT	Reverse
CSD2	CTCTAAGTCAGAGGCTAAGC	Forward
	CGTTTAAGTACATTTTGGTTT	Reverse
CCS	GTGATGGTACTGTCATATGGG	Forward
	CCAAAACTCTCTGTACTTTCA	Reverse
LAC2	GATGGTGAAACCTGGAAAGACG	Forward
	CGCTTCTACGACCGTCAATGT	Reverse
LAC3	TAGCCGCGGGTTATGGCAGC	Forward
	AGCCGGGTCTGTTCTCGGGT	Reverse
LAC4	CCATCTCAAGGTTACAAACTGTCAG	Forward
	CTTCTACCACCGTGAAAATATGGC	Reverse
LAC12	GCCCGATCCGACCCGAAAG	Forward
	ATGAGGGCGGTTTGGCGGTC	Reverse
IPS1 tandem construct	GTTCCCCCTCGGAATCAGCAG	Forward
	TTATGCACTGCCTAGCCGTC	Reverse

miRNA Stem-Loop qRT-PCR primers

	Sequence (5' to 3')	Sense
Reverse Transcription		
miR397-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCATCAA	Reverse
miR398bc-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCAGGGG	Reverse
miR408-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCCAGG	Reverse
miR156a-g	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGTGCTC	Reverse
qRT-PCR		
miR397b	GTGTGCCATTGAGTGCAGCG	Reverse
miR398b/c	TGGGTGTGTTCTCAGGTCG	Reverse
miR408	GTGTGATGCACTGCCTCTTC	Reverse
miR156a-g	ATGCGCTGACAGAAGAGAGT	Reverse
miRuniversal	CCAGTGCAGGGTCCGAGG	Forward