Supplementary Figure S1

Content:

A. nucleotide and amino acid alignment of NtMTP2-X1 and NtMTP2-X2 from *Nicotiana tabacum*

B. nucleotide (ORF with STOP codon) and protein sequence of MTP2 from *Nicotiana tabacum*

C. *MTP2* genes with the highest homology to *NtMTP2*, based on a search through NCBI data base,

D. MTP2 proteins with the highest homology to NtMTP2, based on a search through NCBI data base,

E. comparison of amino acid sequences of NtMTP2 with MTP2 sequences from different species,

F. amino acid alignment of NtMTP2 with NtMTP6.1 and NtMTP 6.2

G. Suggested models of transmembrane topology of chosen MTP2 and MTP6 proteins,

H. Prediction of exons and introns organization in *NtMTP2,*

A. nucleotide and amino acid alignment of NtMTP2-X1 and NtMTP2-X2 from tobacco identified by Papierniak et al.[1]

NtMTP2-X1 has been cloned, characterized and designated as *NtMTP2* in this paper. Sequences were aligned using ClustalW. Differences between two sequences are highlighted by red.

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NtMTP2-X2    ATGGTAGCTAGATTGATTTCAAGCAATCTAAATCGAATACGAAAAACGTATATATCATCA 60
NtMTP2-X1    ATGGTAGCTAGATTGATTTCAAGCAATCTAAATCGAATACGAAAAACGTATATATCATCA 60
             ************************************************************


NtMTP2-X2    ACTATTAGATACAACAACAATTTCACTCCTCTATATCTATCTCCCAATTTCCAATTCGAT 120
NtMTP2-X1    ACTATTAGATACAACAACAATTTCACTCCTCTATATCTATCTCCCAATTTCCAATTCGAT 120
             ************************************************************


NtMTP2-X2    AATGATGATTCAGCTACAAGTATGGGGAGTCGCAGCTTTATGCTTCAGAAAAGATGGCAT 180
NtMTP2-X1    AATGATGATTCAGCTACAAGTATGGGGAGTCGCAGCTTTATGCTTCAGAAAAGATGGCAT 180
             ************************************************************


NtMTP2-X2    ACGGGACATTCACATTCACATCATGACCATGACGACCTCCGCTCCGGTAAAAACGGCGAG 240
NtMTP2-X1    ACGGGACATTCACATTCACATCATGACCATGACGACCTCCGCTCCGGTAAAAACGGCGAG 240
             ************************************************************


NtMTP2-X2    AGGATTTTCCGGCTTGGCCTCGCTGCTGATATTGGCCTCGCCGCCAGTAAAGCTTTCACA 300
NtMTP2-X1    AGGATTTTCCGGCTTGGCCTCGCTGCTGATATTGGCCTCGCCGCCAGTAAAGCTTTCACA 300
             ************************************************************


NtMTP2-X2    GGTTATATATGTGGTAGCACCGCCATTATCGCTGATGCCGCCCATTCTATCTCCGATGTG 360
NtMTP2-X1    GGTTATATATGTGGTAGCACCGCCATTATCGCTGATGCCGCCCATTCTATCTCCGATGTG 360
             ************************************************************


NtMTP2-X2    GTTCTGAGTGGAGTGGCATTGCTGTCGTTTAAAGCTGCAAGGGGTTCCCAAGGACAAAGAA 420
NtMTP2-X1    GTTCTGAGTGGAGTGGCATTGCTGTCGTTTAAAGCTGCAAGGGGTTCCCAAGGACAAAGAA 420
             ************************************************************


NtMTP2-X2    CATCCTTATGGACATGGTAAATTTGAGACTCTTGGAGCTCTTGGAATTTCTGGTGTACTA 480
NtMTP2-X1    CATCCTTATGGACATGGTAAATTTGAGACTCTTGGAGCTCTTGGAATTTCTGGTGTACTA 480
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NtMTP2-X2    TTGGCTACTGCTGGAGGTATTGGATGGCATGCTTTAGATGTTTTGCTGGGATTATGGTCT  540
NtMTP2-X1    TTGGCTACTGCTGGAGGTATTGGATGGCATGCTTTAGATGTTTTGCTGGGATTATGGTCT  540
             ************************************************************


NtMTP2-X2    ACAGCGCCCGAAGTTGTTAACCAGTCATTGAGTCATCTGGACGTGCATGAGCAACATCAT  600
NtMTP2-X1    ACAGCGCCCGAAGTTGTTAACCAGTCATTGAGTCATCTGGACGTGCATGAGCAACATCAT  600
             ************************************************************


NtMTP2-X2    AGTGGACATCACCATGGAATAGATATGGATCACCCTATTCTTGCTTTGAATGTGACTATA  660
NtMTP2-X1    AGTGGACATCACCATGGAATAGATATGGATCACCCTATTCTTGCTTTGAATGTGACTATA  660
             ************************************************************


NtMTP2-X2    CTCTCTATAGCTGTTAAAGAAGGATTATACTGGATGACAAAGAGAGCGGGGGATAAGATT  720
NtMTP2-X1    CTCTCTATAGCTGTTAAAGAAGGATTATACTGGATGACAAAGAGAGCGGGGGATAAGATT  720
             ************************************************************


NtMTP2-X2    GGCAGTGGACTGATGAAAGCCAATGCTTGGCATCATCGTGCTGATGCAGTATCCTCCGTC  780
NtMTP2-X1    GGCAGTGGACTGATGAAAGCCAATGCTTGGCATCATCGTGCTGATGCAGTATCCTCCGTC  780
             ************************************************************


NtMTP2-X2    GTTGCTCTCATAGGAGTT------------------------------------------  798
NtMTP2-X1    GTTGCTCTCATAGGAGTTGGTGGTTCAATCCTTGGGGTGAGGATCCTTGATCCACTTGCT  840
             ******************


NtMTP2-X2    ---------------GGCATGATCATGAAAGCTGGACTTGAAACCGGATATCAGAGTGTC  843
NtMTP2-X1    GGGCTTGTTGTTGCAGGCATGATCATGAAAGCTGGACTTGAAACCGGATATCAGAGTGTC  900
                            *********************************************


NtMTP2-X2    TTGGAATTGGTTGATGCTGCTATTCCTTCACATACCCTGAAGCCTTTCAAGCGCATGATT  903
NtMTP2-X1    TTGGAATTGGTTGATGCTGCTATTCCTTCACATACCCTGAAGCCTTTCAAGCGCATGATT  960
             ************************************************************


NtMTP2-X2    CTACAAGTTGATGGAGTTAAGGGATGCAGTCACCTGAGGGGAAGGAGGGCTGGTTCATAT  963
NtMTP2-X1    CTACAAGTTGATGGAGTTAAGGGATGCAGTCACCTGAGGGGAAGGAGGGCTGGTTCATAT  1020
             ************************************************************


NtMTP2-X2    CTCTATCTTGATGTTATTGTTGAGGTTGACCCCTTTTCTAGTGTGAGTGCCGCACATGAA  1023
NtMTP2-X1    CTCTATCTTGATGTTATTGTTGAGGTTGACCCCTTTTCTAGTGTGAGTGCCGCACATGAA  1080
             ************************************************************


NtMTP2-X2    ATCGGGGAAAACGTCCGCCGTGAAATCCAGCAGTTACATCCCGAAATTGCCGAAGTCTTC  1083
NtMTP2-X1    ATCGGGGAAAACGTCCGCCGTGAAATCCAGCAGTTACATCCCGAAATTGCCGAAGTCTTC  1140
             ************************************************************


NtMTP2-X2    GTACACATAGAGCCATCCACTATACACATTCCACCAACTGTTGTGTTTCAGCAGAGGGCT  1143
NtMTP2-X1    GTACACATAGAGCCATCCACTATACACATTCCACCAACTGTTGTGTTTCAGCAGAGGGCT  1200
             ************************************************************


NtMTP2-X2    AACACAATGGGTGCCCCCCAAGACCATTCTTCAATGGAGCTCGCAGACATTGAAAATAAA  1203
NtMTP2-X1    AACACAATGGGTGCCCCCCAAGACCATTCTTCAATGGAGCTCGCAGACATTGAAAATAAA  1260
             ************************************************************


NtMTP2-X2    GTTTACAACATTTTATCTACAAACTATTCTCAGAAGATGATAATTGAGCGTGTAAATCCA  1263
NtMTP2-X1    GTTTACAACATTTTATCTACAAACTATTCTCAGAAGATGATAATTGAGCGTGTAAATCCA  1320
             ************************************************************


NtMTP2-X2    CATCTGTTGCAAGGACAGATTTTACTTCAAGTTGAAGTTTCTATGCCTCCTGATCTCTTG  1323
NtMTP2-X1    CATCTGTTGCAAGGACAGATTTTACTTCAAGTTGAAGTTTCTATGCCTCCTGATCTCTTG  1380
             ************************************************************


NtMTP2-X2    ATTAGGGATGCGGAAAAAGTTGCAGAAGAAGCAGAGAAATTAATCATAGAGGCAGCACCA  1383
NtMTP2-X1    ATTAGGGATGCGGAAAAAGTTGCAGAAGAAGCAGAGAAATTAATCATAGAGGCAGCACCA  1440
             ************************************************************


NtMTP2-X2    GATATTGTTCATGTGTGCATTCAGCTACGCCTAGGGCAACCTATGCCAGAGCCTTACAAG  1443
NtMTP2-X1    GATATTGTTCATGTGTGCATTCAGCTACGCCTAGGGCAACCTATGCCAGAGCCTTACAAG  1500
             ************************************************************
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2

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NtMTP2-X2   GAATGGGTGGACAGTAGAAGTGAGAGGAAAGATAACTGA         1482
NtMTP2-X1   GAATGGGTGGACAGTAGAAGTGAGAGGAAAGATAACTGA         1539
            ***************************************

NtMTP2-X2   MVARLISSNLNRIRKTYISSTIRYNNNFTPLYLSPNFQFDNDDSATSMGSRSFMLQKRWH 60
NtMTP2-X1   MVARLISSNLNRIRKTYISSTIRYNNNFTPLYLSPNFQFDNDDSATSMGSRSFMLQKRWH 60
            ***********************************************************

NtMTP2-X2   TGHSHSHHDHDDLRSGKNGERIFRLGLAADIGLAASKAFTGYICGSTAIIADAAHSISDV 120
NtMTP2-X1   TGHSHSHHDHDDLRSGKNGERIFRLGLAADIGLAASKAFTGYICGSTAIIADAAHSISDV 120
            ***********************************************************

NtMTP2-X2   VLSGVALLSFKAARVPKDKEHPYGHGKFETLGALGISGVLLATAGGIGWHALDVLLGLWS 180
NtMTP2-X1   VLSGVALLSFKAARVPKDKEHPYGHGKFETLGALGISGVLLATAGGIGWHALDVLLGLWS 180
            ***********************************************************

NtMTP2-X2   TAPEVVNQSLSHLDVHEQHHSGHHHGIDMDHPILALNVTILSIAVKEGLYWMTKRAGDKI 240
NtMTP2-X1   TAPEVVNQSLSHLDVHEQHHSGHHHGIDMDHPILALNVTILSIAVKEGLYWMTKRAGDKI 240
            ***********************************************************

NtMTP2-X2   GSGLMKANAWHHRADAVSSVVALIGV-------------------GMIMKAGLETGYQSV 281
NtMTP2-X1   GSGLMKANAWHHRADAVSSVVALIGVGGSILGVRILDPLAGLVVAGMIMKAGLETGYQSV 300
            **************************               ***************

NtMTP2-X2   LELVDAAIPSHTLKPFKRMILQVDGVKGCSHLRGRRAGSYLYLDVIVEVDPFSSVSAAHE 341
NtMTP2-X1   LELVDAAIPSHTLKPFKRMILQVDGVKGCSHLRGRRAGSYLYLDVIVEVDPFSSVSAAHE 360
            ***********************************************************

NtMTP2-X2   IGENVRREIQQLHPEIAEVFVHIEPSTIHIPPTVVFQQRANTMGAPQDHSSMELADIENK 401
NtMTP2-X1   IGENVRREIQQLHPEIAEVFVHIEPSTIHIPPTVVFQQRANTMGAPQDHSSMELADIENK 420
            ***********************************************************

NtMTP2-X2   VYNILSTNYSQKMIIERVNPHLLQGQILLQVEVSMPPDLLIRDAEKVAEEAEKLIIEAAP 461
NtMTP2-X1   VYNILSTNYSQKMIIERVNPHLLQGQILLQVEVSMPPDLLIRDAEKVAEEAEKLIIEAAP 480
            ***********************************************************

NtMTP2-X2   DIVHVCIQLRLGQPMPEPYKEWVDSRSERKDN         493
NtMTP2-X1   DIVHVCIQLRLGQPMPEPYKEWVDSRSERKDN         512
            *******************************
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B. nucleotide and protein sequence of MTP2 from *Nicotiana tabacum*

START and STOP sequence are underlined.

>*NtMTP2* (1539bp - XM_016593653.1); initially identified by Papierniak et al.[1] as NtMTP2-X1

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ATGGTAGCTAGATTGATTTCAAGCAATCTAAATCGAATACGAAAAACGTATATATCATCAACTATTAGAT
ACAACAACAATTTCACTCCTCTATATCTATCTCCCAATTTCCAATTCGATAATGATGATTCAGCTACAAG
TATGGGGAGTCGCAGCTTTATGCTTCAGAAAAGATGGCATACGGGACATTCACATTCACATCATGACCAT
GACGACCTCCGCTCCGGTAAAAACGGCGAGAGGATTTTCCGGCTTGGCCTCGCTGCTGATATTGGCCTCG
CCGCCAGTAAAGCTTTCACAGGTTATATATGTGGTAGCACCGCCATTATCGCTGATGCCGCCCATTCTAT
CTCCGATGTGGTTCTGAGTGGAGTGGCATTGCTGTCGTTTAAAGCTGCAAGGGTTCCCAAGGACAAAGAA
CATCCTTATGGACATGGTAAATTTGAGACTCTTGGAGCTCTTGGAATTTCTGGTGTACTATTGGCTACTG
CTGGAGGTATTGGATGGCATGCTTTAGATGTTTTGCTGGGATTATGGTCTACAGCGCCCGAAGTTGTTAA
CCAGTCATTGAGTCATCTGGACGTGCATGAGCAACATCATAGTGGACATCACCATGGAATAGATATGGAT
CACCCTATTCTTGCTTTGAATGTGACTATACTCTCTATAGCTGTTAAAGAAGGATTATACTGGATGACAA
AGAGAGCGGGGGATAAGATTGGCAGTGGACTGATGAAAGCCAATGCTTGGCATCATCGTGCTGATGCAGT
ATCCTCCGTCGTTGCTCTCATAGGAGTTGGTGGTTCAATCCTTGGGGTGAGGATCCTTGATCCACTTGCT
GGGCTTGTTGTTGCAGGCATGATCATGAAAGCTGGACTTGAAACCGGATATCAGAGTGTCTTGGAATTGG
TTGATGCTGCTATTCCTTCACATACCCTGAAGCCTTTCAAGCGCATGATTCTACAAGTTGATGGAGTTAA
GGGATGCAGTCACCTGAGGGGAAGGAGGGCTGGTTCATATCTCTATCTTGATGTTATTGTTGAGGTTGAC
CCCTTTTCTAGTGTGAGTGCCGCACATGAAATCGGGGAAAACGTCCGCCGTGAAATCCAGCAGTTACATC
CCGAAATTGCCGAAGTCTTCGTACACATAGAGCCATCCACTATACACATTCCACCAACTGTTGTGTTTCA
GCAGAGGGCTAACACAATGGGTGCCCCCCAAGACCATTCTTCAATGGAGCTCGCAGACATTGAAAATAAA
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GTTTACAACATTTTATCTACAAACTATTCTCAGAAGATGATAATTGAGCGTGTAAATCCACATCTGTTGC
AAGGACAGATTTTACTTCAAGTTGAAGTTTCTATGCCTCCTGATCTCTTGATTAGGGATGCGGAAAAAGT
TGCAGAAGAAGCAGAGAAATTAATCATAGAGGCAGCACCAGATATTGTTCATGTGTGCATTCAGCTACGC
CTAGGGCAACCTATGCCAGAGCCTTACAAGGAATGGGTGGACAGTAGAAGTGAGAGGAAAGATAACTGA
```

>NtMTP2 (512aa - XP_016449139.1)

```
MVARLISSNLNRIRKTYISSTIRYNNNFTPLYLSPNFQFDNDDSATSMGSRSFMLQKRWHTGHSHSHHDH
DDLRSGKNGERIFRLGLAADIGLAASKAFTGYICGSTAIIADAAHSISDVVLSGVALLSFKAARVPKDKE
HPYGHGKFETLGALGISGVLLATAGGIGWHALDVLLGLWSTAPEVVNQSLSHLDVHEQHHSGHHHGIDMD
HPILALNVTILSIAVKEGLYWMTKRAGDKIGSGLMKANAWHHRADAVSSVVALIGVGGSILGVRILDPLA
GLVVAGMIMKAGLETGYQSVLELVDAAIPSHTLKPFKRMILQVDGVKGCSHLRGRRAGSYLYLDVIVEVD
PFSSVSAAHEIGENVRREIQQLHPEIAEVFVHIEPSTIHIPPTVVFQQRANTMGAPQDHSSMELADIENK
VYNILSTNYSQKMIIERVNPHLLQGQILLQVEVSMPPDLLIRDAEKVAEEAEKLIIEAAPDIVHVCIQLR
LGQPMPEPYKEWVDSRSERKDN*
```

C. *MTP2* genes with the highest homology to *NtMTP2*, based on a search through NCBI data base

The cDNA nucleotide sequence acc no XM_016593653.1 (named later in this study as *NtMTP2*) was used as a query to search through NCBI data base for genes with the highest homology (https://blast.ncbi.nlm.nih.gov/)

The list of genes identified as the best Blast Hits are given in the Table below

Description	Identity	Max score	Total score	Query cover	E-value	Accession no.	References
Nicotiana sylvestris metal tolerance protein 2	99.68%	2815	2815	100%	0.0	XM_009782572.1	NCBI
Nicotiana attenuata metal tolerance protein 2, transcript variant X2	98.70%	2732	2732	100%	0.0	XM_019378745.1	NCBI
Nicotiana tomentosifornis metal tolerance protein 2, transcript variant X2	97.26%	2599	2599	99%	0.0	XM_009606364.1	NCBI
Solanum tuberosum metal tolerance protein 2, transcript variant X1	85.56%	1544	1544	98%	0.0	XM_006359348	Leitch et al. 2008 [2]
Glycine max Metal tolerance protein 2	74.90%	653	718	74%	0.0	XM_003518906.4	NCBI
Cucumis sativus Metal tolerance protein 2	73.37%	557	557	74%	3e-157	XM_004136132.3	NCBI
Populus trichocarpa Metal tolerance protein 2	71.44%	645	645	82%	0.0	XM_006386701.2	Gustin et al. 2011 [3]
Oryza sativa Japonica Group Metal tolerance protein 2	71.09%	442	442	59%	4e-121	XP_015628702.1	Gustin et al. 2011 [3]
Vitis vinifera Metal tolerance protein 2	61.75%	497	497	99%	1e-171	XP_002269478.3	Shirazi et al. 2019 [4]

| *Medicago truncatula* Metal tolerance protein 2 isoform X1 | 61.21% | 475 | 475 | 96% | 3e-164 | XP_013469204.1 | León-Mediavilla, et al. 2018 [5] |

The sequences of *NtMTP6.1* and *NtMTP6.2* genes were not annotated in the NCBI; they were extracted from the genome annotation files at Sol Genomics (Liu et al. 2019). The identity of nucleotide sequences of *NtMTP6.1* and *NtMTP6.2* with *NtMTP2* is given below.
Comparison of amino acid sequences is given in the section (d) and (e) of this file.

| NtMTP6.1 | 98.39% | - | - | - | Nitab4.5_0001447g0040.1 | Liu et al. 2019 [6] |
| NtMTP6.2 | 96.83% | - | - | - | Nitab4.5_0001697g0140.1 | Liu et al. 2019 [6] |

D. MTP2 proteins with the highest homology to NtMTP2, based on based on a search through NCBI data base (https://blast.ncbi.nlm.nih.gov/)

Description	Ident.	Max score	Total score	Query cover	E-value	Accession no.
Nicotiana sylvestris metal tolerance protein 2	99,41%	1041	1041	100%	0.0	XP_009780874.1
Nicotiana attenuata metal tolerance protein 2 isoform X1	97.85%	1023	1023	100%	0.0	XP_019234290.1
Nicotiana tomentosifornis metal tolerance protein 2 isoform X2	95.50%	945	945	99%	0.0	XP_009604659.1
Solanum tuberosum metal tolerance protein 2 isoform X1	82.42%	799	799	99%	0.0	XP_006359410.1
Cucumis sativus metal tolerance protein 2	65.26%	591	591	91%	0.0	XP_004136180.1
Populus trichocarpa metal tolerance protein 2	65.71%	558	558	87%	0.0	XP_006386763.1
Glycine max metal tolerance protein 2	62.88%	557	557	95%	0.0	XP_003536229.1
Vitis vinifera metal tolerance protein 2	61.67%	570	570	98%	0.0	XP_002269478.3
Medicago truncatula metal tolerance protein 2 isoform X1	60.37%	555	555	95%	0.0	XP_013469204.1
Cation efflux family protein [*Arabidopsis thaliana*] MTP6	60.27%	533	533	85%	0.0	NP_182304.2
Oryza sativa Japonica Group metal tolerance protein 2	59.81%	485	485	81%	1e-167	XP_015628702.1

E. Comparison of amino acid sequences of NtMTP2 with MTP2 sequences from different species;

Sequence identity between selected MTP proteins from *N. tabacum, A. thaliana*, *V. vinifera*, *P. trichocarpa, Cucumis staivus, O. sativa. M. trunculata*, *S. tuberosum* species. The list of MTP2 proteins and percent identity matrix – created by Clustal2.1. MTP proteins chosen for comparison reside on the same clade on the dendrogram depicted in the Figure 1.

	StMTP2	NtMTP6.1	NtomMTP2	NtMTP6.2	NaMTP2	NtMTP2	NsMTP2	AtMTP6	OsMTP2	PtrMTP6	VvMTP2	CsMTP2	GmMTP2	MtMTP6	MtMTP2	AtMTP2
StMTP2	100.00															
NtMTP6.1	82.53	100.00														
NtomMTP2	84.77	93.63	100.00													
NtMTP6.2	84.60	93.75	98.83	100.00												
NaMTP2	85.54	96.51	96.88	96.48	100.00											
NtMTP2	84.34	98.36	95.31	94.92	97.85	100.00										
NsMTP2	84.34	97.74	95.12	94.73	97.66	99.41	100.00									
AtMTP6	59.15	58.43	60.35	60.22	60.35	59.69	59.69	100.00								
OsMTP2	60.16	59.04	61.60	61.44	61.60	60.80	60.80	62.88	100.00							
PtrMTP6	62.14	59.07	60.81	60.89	61.21	60.40	60.40	62.18	62.79	100.00						
VvMTP2	62.50	57.65	61.14	57.88	62.30	61.11	61.31	66.67	67.20	68.94	100.00					
CsMTP2	62.63	61.03	62.91	62.99	63.11	62.30	62.30	65.64	63.93	67.94	68.27	100.00				
GmMTP2	63.83	61.57	64.51	64.38	64.30	63.26	63.05	65.26	67.12	67.42	69.47	71.66	100.00			
MtMTP6	62.82	59.91	61.86	62.14	62.27	61.24	61.24	62.78	63.37	65.72	66.60	68.02	80.94	100.00		
MtMTP2	20.86	21.29	20.97	20.70	20.97	21.58	21.58	20.79	19.75	22.26	21.15	23.10	20.56	19.94	100.00	
AtMTP2	23.05	22.85	22.73	22.67	23.43	23.78	23.43	23.08	22.42	23.43	23.59	26.57	25.35	24.11	50.46	100.00

F. Amino acid sequence alignment of NtMTP2 and NtMTP6.1 and NtMTP6.2;

Sequences were aligned using ClustalW; asterisk indicate sequence identity, the cation diffusion facilitator (CDF) signature sequence is marked in the frame. Histidine residues are highlighted by black, leucine from LZ-motif are black, whereas the transmembrane domains (TMD) predicted by TMPpred are grey shading.

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CLUSTAL O(1.2.4) multiple sequence alignment

NtMTP6.1     MTLFDSQKELLRNQIDQLENIVLRQCNLTGVNPLSQEMAAGALSIKIGKRPRDLLNPKAI     60
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     MTLFDSQKELLRNQIDQLENIVLRQCNLTGVNPLSQEMAAGALSIKIGKRPRDLLNPKAI     60


NtMTP6.1     KYMQSVFSIKDSINKKETREISALFGVTVTQVRDFFTAQRTRVRKFLRLSREKASISNAS     120
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     KYMQSVFSIKDAINKKETREISALFGVTVTQVRDFFTAQRTRVRKFLRLSREKASISNAF     120


NtMTP6.1     IEGPCPIPLSSESDPSSQTEPVPLDSVAPTCTEEGPSCSTQDDVLTGIEETDKHFLDNIL     180
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     IEGPCPIPLSS—DPSSHTEPVPLDSVAPTCTDVGPSCSTQDEVLTGIEETDKHFLDNIL     178


NtMTP6.1     TLMRKEETFSGQVKLMDWILEVQNPSVLFWA-----------------------------     211
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     TLMRKEETFSGQVKLMDWILEVQNPSVLFWFLAKGGVMILATWLSQAAVEEQTGVLHIIL     238


NtMTP6.1     -----------------------------------RILLAKWSKMFAKSQAMKKRNG     233
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     KVLCHLPLHKAFPVHMSAILQSVNRLRFYRTPDISNRARILLAKWSKMFAKSQAMKKRNG     298


NtMTP6.1     IKSASDVQDELLLQQSIGEVMGDEIWNSKAEDVGESHANLCGPSEYSRKLDSSHPVKLLT     293
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     IKSASDVQDELLLQQSISEVMGDEIWNSKAEDVGESHANLCGPSENSSAL----------     348


NtMTP6.1     ASSDDSTKRLNKGALASSILRCLCATSETRERRKVQLMEQPSQRTTGRSLAVGRPATATQ     353
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     -----------------------ATSETRERRKVQLMEQPSQRTTGRSLAVGRPATATQ     384


NtMTP6.1     GRPLSADDIQKAKMRAQFMQSKYGKAKNDESSRVKAEAPNGVTTPQDDILQGDPKLLGCP     413
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     GRPLSADDIQKAKMRAQFMQSKYGKAKNDESSLVKPEAPNGVTSPQDDILQGAPKLQGCP     444


NtMTP6.1     KDDEHEKLDSVALKGFKQQESHRKLSFDVEEPPWKRCRRMQIPWCKPPEVKMSDAWKVCD     473
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     KDDEHEKLDSVALKGSNQQESHRKLSFDVEEPPWKRCRRMQIPWCKPPEVTMSDAWKVCA     504


NtMTP6.1     GGESKEVDIQNNRIRRERETIYRTVQEIPLNPKEPWDREMDPDDTLTTEIPIEQLPDAEG     533
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     GGESKEVDIQNKRIRRERETIYRTVQEIPLNPKEPWDREMDPDDTLTTEIPIEQLPDTEG     564


NtMTP6.1     AETVVLRPEDEETEAASASTSNGIATTAEPDVELLAVLLKNPELVYALTSGQAGNLSSEE     593
NtMTP2       ------------------------------------------------------------     0
NtMTP6.2     AETVVLRPEDEETEAASASTSNGIATTAEPDVELLAVLLKNPELVYALTSGQAGNLSSEE     624
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NtMTP6.1    TVKLLDMIKANGMNSLSSVTDLGRTAEKKVEVSLPSPTPSSDPGTSGSMQGFAKNPFSQR    653
NtMTP2      ------------------------------------------------------------    0
NtMTP6.2    TVKLLDMIKANGMNSLNSVTDLGRIAEKKVEVSLPSPTPSSDPGTSGSMQNFAKNPFSQR    684


NtMTP6.1    SLMAVPEANGATQLAVLVRSQEKLQTSSSIYPQSTSSTMLAPQQLPIAPQLAQQLSLLQA    713
NtMTP2      ------------------------------------------------------------    0
NtMTP6.2    SLMAVPEANGATRLAGLVRSQEKLQASSSIYPQSTSTTMLASQQLPIAPQLAQQLSLLQA    744


NtMTP6.1    AAGSFGKDHRLSPLNPNLNQTVLANPMHSQLSASEPAVNRNNYSPFGLTEYNLHSATASA    773
NtMTP2      ------------------------------------------------------------    0
NtMTP6.2    AAGSFEKDHRPSPLNPSLNQTALANPMHSQLSTSEPAVNRNNYSPFGLTEYNLHSAT-A    802


NtMTP6.1    ATTRIQGQTSGNIRSSPMPIANVQERTISLHMPQMAVSHTPPRPQLQTQPRPGYAPEHMW    833
NtMTP2      ------------------------------------------------------------    0
NtMTP6.2    ATTRIQGETSGNIRSSPMPIANVQQRTVSLHMPQMAVSHTPPRPQLQTQPQPGYTPEHMW    862


NtMTP6.1    GTMSGSTLNRGYQENSIPNHYNTRLAGHGEPGLQQAAWRGNYVEEAGFESWSPDNSPVRR    893
NtMTP2      ------------------------------------------------------------    0
NtMTP6.2    GTMSGSALNRGYQENSIPNHYNAHLAGHVEPGLQHAAWRGNYVEEAGFESWSPDNSPVRR    922


NtMTP6.1    QEQLGRWNHSEPRMNMRENYRSDWSTLRNPSYYSGYHGPDDGGNRRWA------------    941
NtMTP2      ------------------------------------------------------------    0
NtMTP6.2    QEQVGRWNHSEHRMNMRENYRSDLSTSRNPSYHSGYRGPDDGGNRRWVAFFNHSSSASQI    982


NtMTP6.1    ---------------RLISSNLNRIRKTYISSTIRYNNNFTPLYLSPNFQFDNDDSATSM    986
NtMTP2      -----------MVARLISSNLNRIRKTYISSTIRYNNNFTPLYLSPNFQFDNDDSATSM    48
NtMTP6.2    RAILPNFIELLNMVARFISSNLNRIRKTYIASTIRYNNNFTPLYLSPNLQFDNDDSATSI    1042
                          *:***************:***************:*********:.
```
                                                            TMD I
```
NtMTP6.1    GSRSFMLQKRWHTGHSHSHHDHDDLRSGKNGERIFRLGLAADIGLAASKAFTGYICGSTA    1046
NtMTP2      GSRSFMLQKRWHTGHSHSHHDHDDLRSGKNGERIFRLGLAADIGLAASKAFTGYICGSTA    108
NtMTP6.2    GNRSFMLQKRWHMGHSHSHHDHDDLRSGKDGERIFRLGLAADIGLAASKAFTGYICGSTA    1102
            * .********  ***********  :************************ ***  ***
```
       HxxxD   **TMD II**   **TMD III**
```
NtMTP6.1    IIADAAHSISDVVLSGVALLSFKAARVPKDKEHPYGHGKFETLGALGISGVLLATAGGIG    1106
NtMTP2      IIADAAHSISDVVLSGVALLSFKAARVPKDKEHPYGHGKFETLGALGISGVLLATAGGIG    168
NtMTP6.2    IIADAAHSISDVVLSGVALLSFKAARVPKDKEHPYGHGKFETLGALGISGVLLATAGGIG    1162
            ************************************************************
```
                                                            TMD IV
```
NtMTP6.1    WHALDVLLGLWSTAPEVVNQSLSHLDVHEQHHSGHHHGIDMDHPILALNVTILSIAVKEG    1166
NtMTP2      WHALDVLLGLWSTAPEVVNQSLSHLDVHEQHHSGHHHGIDMDHPILALNVTILSIAVKEG    228
NtMTP6.2    WHALDVLLGLWSTAPEVVNQSLSHLDVHEQHHSGHHHGIDMDHPILALNVTILSIAVKEG    1222
            ************************************************************
```
                                          TMD V
```
NtMTP6.1    LYWMTKRAGDKIGSGLMKANAWHHRADAVSSVVALIGVGGSILGVRILDPLAGLVVAGMI    1226
NtMTP2      LYWMTKRAGDKIGSGLMKANAWHHRADAVSSVVALIGVGGSILGVRILDPLAGLVVAGMI    288
NtMTP6.2    LYWITKRAGDKIGSGLMKANAWHHRADAVSSVVALVGVGGSILGVRILDPLAGLVVAGMI    1282
            ***:***************************    :********************  ***
```
       LZ-motif
```
NtMTP6.1    MKAGLETGYQRSEICL--------------------------------------------    1242
NtMTP2      MKAGLETGYQSVLELVDAAIPSHTLKPFKRMILQVDG-----------------------    325
NtMTP6.2    MKAGLETGYQSVLELVDAAIPSHTLKPFKRTILQVDGVKATIPFSHLLDVDTVVRNSLLP    1342
            **********        :
```
                                    ZT-dimer
```
NtMTP6.1    ----------------KMGRGCSHLRGRRAGSYLYLDVIVEVDPFSSVSAAHEIGENVRR    1286
NtMTP2      ----------------VKGCSHLRGRRAGSYLYLDVIVEVDPFSSVSAAHEIGENVRR    367
NtMTP6.2    PTCQHLIMYADEIFEKHGLTGCSHLRGRRAGSYLYLDVIVEVDPFSSVSAAHEIGENVRR    1402
                            ******************************************
```

```
NtMTP6.1    EIQQLHPEIAEVFVHIEPSTIHIPPTVVFQQRANTMGAPQDHSSMELADIENKVYNILST    1346
NtMTP2      EIQQLHPEIAEVFVHIEPSTIHIPPTVVFQQRANTMGAPQDHSSMELADIENKVYNILST    427
```

```
NtMTP6.2      EIQQLHPEIAEVFVHIEPSLIHIPPTVVFQQKANTMCGPQDHSAMELADIENIVYNIFST  1462
              ******************* ************:****  .*****:******* ****:**


NtMTP6.1      NYSQKMIIERVNPHLLQGQILLQVEVSMPPDLLIRDAEKVAEEAEKLIIEAAPDIVHVCI  1406
NtMTP2        NYSQKMIIERVNPHLLQGQILLQVEVSMPPDLLIRDAEKVAEEAEKLIIEAAPDIVHVCI  487
NtMTP6.2      NFSQKMIIERVTPHLLQGQILLQVEVSMPPDLLIRDAGKVAKEAEKLIIEAAPDIVHVSI  1522
              *:********* .**************************  ***:**************** .*


NtMTP6.1      QLRLGQPMPEPYKEWVDSRSERKDN---  1431
NtMTP2        QLRLGQPMPEPYKEWVDSRSERKDN---  512
NtMTP6.2      QLRLGQPMPEPYKELVDSRSGRKDPNQI  1550
              ************** ***** ***
```

G. Suggested models of transmembrane topology of chosen MTP2 and MTP6 proteins; membrane-spanning regions and their orientation. The algorithm was based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins. The prediction was made using a combination of several weight-matrices for scoring.

Protein name	TmPred prediction
NtMTP2 XP_016449139.1	-----> STRONGLY prefered model: N-terminus inside 5 strong transmembrane helices, total score : 5342 # from to length score orientation 1 91 110 (20) 688 i-o 2 122 140 (19) 664 o-i 3 158 174 (17) 1737 i-o 4 215 235 (21) 551 o-i 5 261 281 (21) 1702 i-o
NtomMTP2 XP_009604659.1	-----> STRONGLY prefered model: N-terminus inside 5 strong transmembrane helices, total score : 5386 # from to length score orientation 1 93 112 (20) 688 i-o 2 124 142 (19) 664 o-i 3 160 176 (17) 1737 i-o 4 217 240 (24) 601 o-i 5 263 283 (21) 1696 i-o
AtMTP6 At2g4783	-----> STRONGLY prefered model: N-terminus inside 5 strong transmembrane helices, total score : 5686 # from to length score orientation 1 94 113 (20) 678 i-o 2 112 135 (24) 593 o-i 3 152 168 (17) 1599 i-o 4 196 217 (22) 882 o-i 5 242 262 (21) 1934 i-o

PtrMTP6 Potri.T034500	-----> STRONGLY prefered model: N-terminus inside 5 strong transmembrane helices, total score : 5766 # from to length score orientation 1 98 119 (22) 552 i-o 2 130 153 (24) 767 o-i 3 170 186 (17) 1793 i-o 4 223 248 (26) 705 o-i 5 273 293 (21) 1949 i-o
VvMTP2	-----> STRONGLY prefered model: N-terminus inside 3 strong transmembrane helices, total score : 3978 # from to length score orientation 1 129 150 (22) 608 i-o 2 201 217 (17) 1559 o-i 3 304 324 (21) 1811 i-o
GmMTP2 XP_003536229.1	-----> STRONGLY prefered model: N-terminus inside 5 strong transmembrane helices, total score : 5300 # from to length score orientation 1 80 100 (21) 517 i-o 2 115 133 (19) 735 o-i 3 151 167 (17) 1661 i-o 4 207 228 (22) 543 o-i 5 254 274 (21) 1844 i-o
CsMTP2 XP_004136180.1	-----> STRONGLY prefered model: N-terminus inside 5 strong transmembrane helices, total score : 5215 # from to length score orientation 1 84 105 (22) 531 i-o 2 111 134 (24) 550 o-i 3 156 172 (17) 1643 i-o 4 215 235 (21) 647 o-i 5 261 281 (21) 1844 i-o
AtMTP2 At3g61940	-----> STRONGLY prefered model: N-terminus inside 6 strong transmembrane helices, total score : 11980 # from to length score orientation 1 33 50 (18) 2007 i-o 2 66 85 (20) 1592 o-i 3 100 119 (20) 1920 i-o 4 136 157 (22) 2714 o-i 5 200 221 (22) 1571 i-o 6 227 245 (19) 2176 o-i
MtMTP2 Medtr4g064893.1	-----> STRONGLY prefered model: N-terminus inside 6 strong transmembrane helices, total score : 11842 # from to length score orientation 1 56 75 (20) 2037 i-o 2 89 108 (20) 1745 o-i 3 126 146 (21) 2201 i-o 4 159 177 (19) 2436 o-i

5	263	282 (20)	1439 i-o
6	286	306 (21)	1984 o-i

H. Prediction of exons and introns organization in *NtMTP2*.

FGENESH tools were used to identify ORF sequence with Start Side of Transcription (TSS), sizes/length/position of introns and exons in the regions of the CoDing Sequences (CDS; CDSf- First CoDing Sequence, CDSi- Inner CoDing Sequence, CDSl- Last CoDing Sequence) and poliA region (polA).
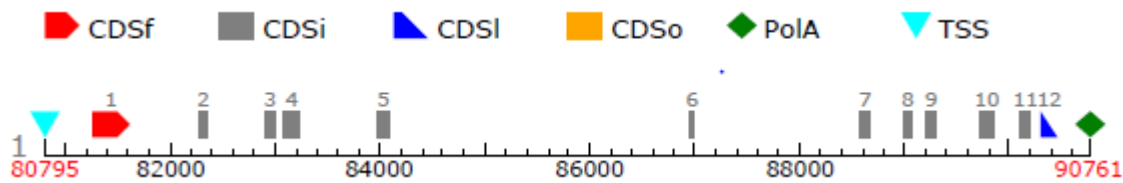
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