Supplementary material

Overexpression of calmodulin-like (*ShCML44*) stress-responsive gene from *Solanum habrochaites,* enhances tolerance to multiple abiotic stresses

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Table S1. List of primer sequences.

Gene	Forward primer	Reverse primer
For gene amplification and qRT-PCR		
ShCML	TCATGTCTCCGATCAACTCAAT	TTGGTAGATTGTAAGCGGGATT
ß-actin	ATGGCAGACGGAGAGGATATTCA	AGACGGAGAATGGCATGTGG
EF1a	CGTGGTTATGTTGCCTCAAA	ACAGCAATGTGGGAAGTGTG
For promoter amplification		
PCML	TCGGAGGCAAAATTAAGGAA	TGACGCGAAATAATGATGCT
For transgenic plants identification		
ShCML	TCATGTCTCCGATCAACTCAAT	TTGGTAGATTGTAAGCGGGATT
CaMV35S	ACGCACAATCCCACTATCCTTC	
For qRT-PCR		
CML	ATGTCTCCGATCAACTCAAT	TACCAAGTAGCAACTCTAGC
SOD	TTTCTGGGAAGCAAATCGTC	TTGGGCAATGAAGAAGAAGC
Peroxidase	CTTGCCCTAATGCTCTCACC	GCATCACAACCCTGAACAAA
GST	GCAAGCCCATTTGTGAGTCT	TGCTGACCCCTTATCATCG
LOX	TCATTTTCCCCTGGCAAGTA	TGGTGCATTTGGATCTTCCT
PR1	GGATTGACACCAGCAGAATC	CCATGTAACCCCAATGCCTA
PR2	ATGGGCTGAAGGATCAGTTG	GACACTCTTCTTGAACTTTGTTGG
Catalase	TCCCAGTTAATGCTCCCAAG	CTCAGCAGGACGACAAGGAT
sucrose synthase	GTGGAGACCGAAGAAAGGAA	GGGAAGAAATCCATCGGAAC
β-amylase	GCATTCGTGCCAACTTATCTT	GATTTCCACTCCGTGCTTTC
Hsp90-1	GCACTTCTCTGTTGAAGGTCAG	ATGAACACACGGCGAACATA
ERD1	CAGAACTGGCCTCAAGCAC	AGCATGGCAGATTCAGATCC
ERD2	ATTTTGAGTGGCGAGGGTAA	TTGGGATAGTCGTGTTTCTGG

Supplementary Figures legend

Figure S1. Alignment of *ShCML44* nucleotide sequence with sequences from *S. lycopersicum* (*SlCML44*) and *S. pennellii* (*SpCML44*). Multiple sequence alignments were conducted using ClustalW2.

Figure S2. Phylogenetic analysis of *Solanum habrochaites* (*ShCML44), S. lycopersicum* (*SlCML44*) and *S. pennellii* (*SpCML44*) proteins with other species CML proteins.

Figure S3. *ShCML44* overexpression plants response towards drought tolerance in tomato. Comparison of plant height (a), number of leaves per plant (b), leaf length (c) leaf width (d), of transgenic and wild-type plants under normal and drought stress conditions.

Figure S1

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SlCML           ATGTCTCCGATCAACTCAATTAATTTGTCAAAAATCTTCTCAAAGCTTGACAAGAATGGT
SpCML           ATGTCTCCGATCAACTCAATTAATTTGTCAAAGATCTTCTCAAAGCTTGACAAGAATGGT
ShCML           ATGTCTCCGATCAACTCAATTAATTTGTCAAAAATCTTCTCAAAGCTTGACAAGAATGGT
                **********************************.**************************

SlCML           GATGGCCTTGTGTGTCTTGATGAGTTAAAGGGATTTCTTGATACAATAGGAATTATTGCA
SpCML           GATGGCCTTGTGTGTCTTGATGAGTTAAAGGGATTTCTTGATACAATAGGAATTATTGCA
ShCML           GATGGCCTTGTGTGTCTTGATGAGTTAAAGGGATTTCTTGATACAATAGGAATTATTGCA
                ************************************************************

SlCML           AGCCAAGAGGAGCTAGAGTTGCTACTTGGTAAAACAAGCCTAGACTCCATTGATTTTTTC
SpCML           AGCCAAGAGGAGCTAGAGTTGCTACTTGGTAAAACAAGCCTAGACTTCATTGATTTTTTC
ShCML           AGCCAAGAGGAGCTAGAGTTGCTACTTGGTAAAACAAGCCTAGACTCCATTGATTTTTTC
                ********************************************* *************

SlCML           TTCTTCTATGATGCTATCACAAAGGCAAATATTAATAAAGGTAGCAATTATAAGCACGAG
SpCML           TTCTTCTATGATGCTATCACAAAGGCAAATATTAATAAAGGTAGCAATTATAAGCACGAG
ShCML           TTCTTCTATGATGCTATCACAAAGGCAAATATTAATAAAGGTAGCAATTATAGACACGAG
                ****************************************************..******

SlCML           GATCGAGAAAATGTTTTCTTGGAAAATGACCTACGTAAAGTGTTTAGAGTATTCGATTTA
SpCML           GATCGAGAAAATGTTTTCTTGGAAAATGACCTACGTAAAGTGTTTAGAGTATTCGATTTA
ShCML           GATCGAGAAAATGTTTTCTTGGAAAATGACCTACGTAAAGTGTTTAGAGTATTCGATTTA
                ************************************************************

SlCML           AACGAGGACGGATTCATATGTTGTGAGGAGCTGCAAAGAGCATTGTCAAGATTAGGATTG
SpCML           AACGAGGACGGATTCATATGTTGTGAGGAGCTGCAAAGAGCATTGTCAAGATTAGGATTG
ShCML           AACGAGGACGGATTCATATGTTGTGAGGAGCTGCAAAGAGCATTGTCAAGATTAGGATTG
                ************************************************************

SlCML           TGGGATGAACAATGTGGGAAAGATTGTAAGAGTATGATCAATGTTTATGACAAAAATTTA
SpCML           TGGGATGAACAATGTGGGAAAGATTGTAAGAGTATGATCAATGTTTATGACAAAAATTTA
ShCML           TGGGATGAACAATGTGGGAAAGATTGTAAGAGTATGATCAATGTTTATGACAAAAATTTA
                ************************************************************

SlCML           GATGGAAAACTTGATTATGAGGAGTTTAAAGACATGATGTTTGATAATTAA
SpCML           GATGGAAAACTTGATTATGAGGAGTTTAAAGACATGATGTTTGATAATTAA
ShCML           GATGGAAAACTTGATTATGAGGAGTTTAAAGACATGATGTTTGATAATTAA
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Figure S2

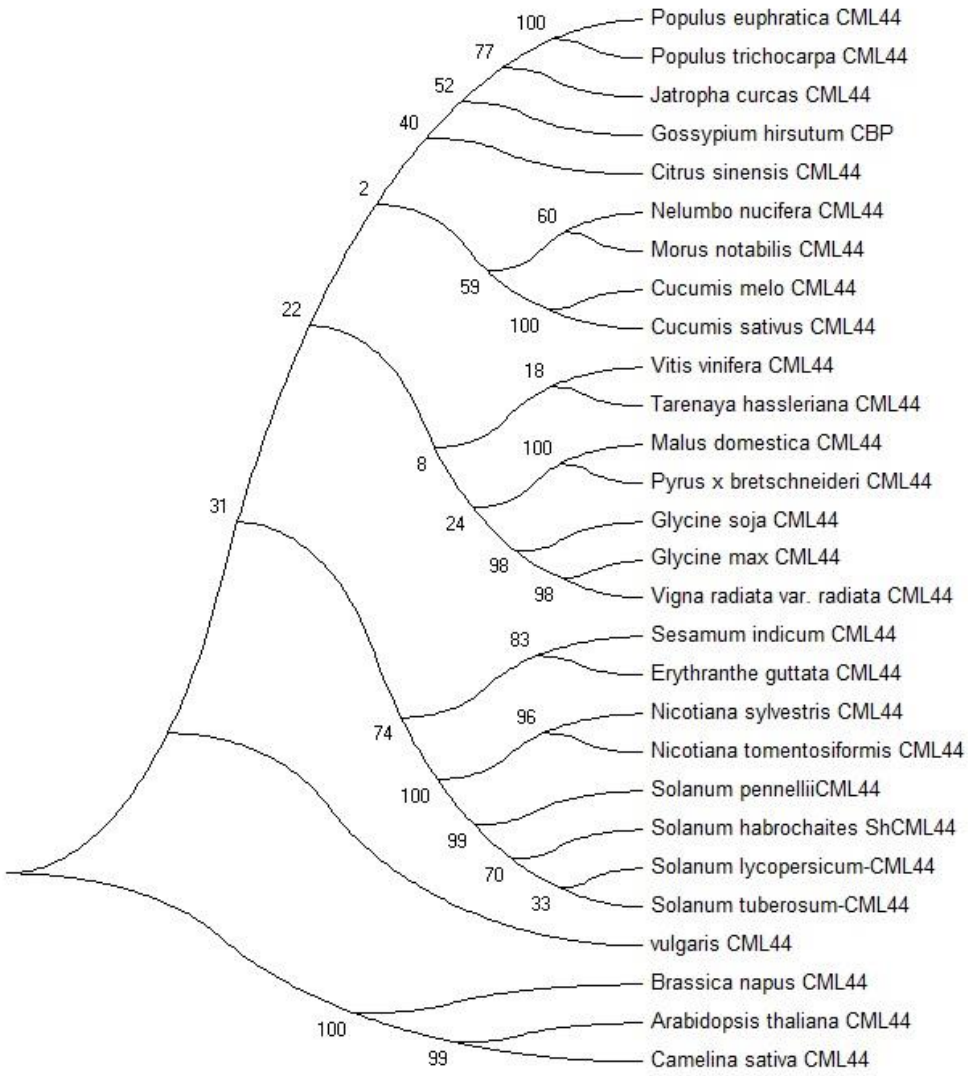
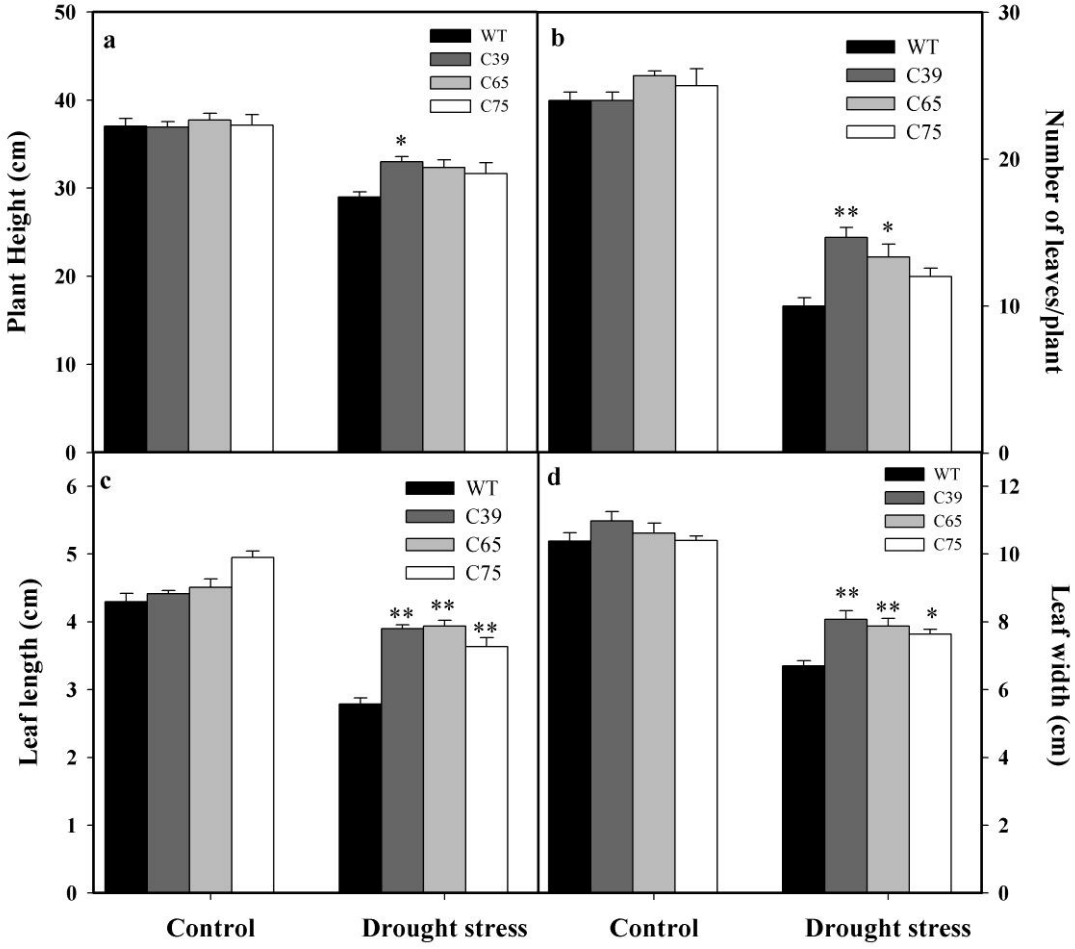

Populus euphratica CML44
Populus trichocarpa CML44
Jatropha curcas CML44
Gossypium hirsutum CBP
Citrus sinensis CML44
Nelumbo nucifera CML44
Morus notabilis CML44
Cucumis melo CML44
Cucumis sativus CML44
Vitis vinifera CML44
Tarenaya hassleriana CML44
Malus domestica CML44
Pyrus x bretschneideri CML44
Glycine soja CML44
Glycine max CML44
Vigna radiata var. radiata CML44
Sesamum indicum CML44
Erythranthe guttata CML44
Nicotiana sylvestris CML44
Nicotiana tomentosiformis CML44
Solanum pennelliiCML44
Solanum habrochaites ShCML44
Solanum lycopersicum-CML44
Solanum tuberosum-CML44
vulgaris CML44
Brassica napus CML44
Arabidopsis thaliana CML44
Camelina sativa CML44
100
77
52
40
2
60
59
100
22
18
8
100
24
98
98
31
83
74
96
100
99
70
33
100
99

Figure S3


a
b
c
d
WT
C39
C65
C75
Plant Height (cm)
Number of leaves/plant
Leaf length (cm)
Leaf width (cm)
Control
Drought stress
Control
Drought stress