

Supplementary Information

Table S1. The members of NAD-ME family in *Populus trichocarpa*.

JGI gene name	Assigned name	Location	Amino acid number	Protein ID
estExt_fgenesh4_pg.C_LG_II1218	<i>PtNADP-ME1</i>	LG_II:10105588-10114861	627	816506
eugene3.00400157	<i>PtNADP-ME2</i>	scaffold_40:1161255-1170200	627	591812
eugene3.00021450	<i>PtNADP-ME3</i>	LG_II:11890404-11897010	607	552135
estExt_Genewise1_v1.C_LG_XIV1110	<i>PtNADP-ME4</i>	LG_XIV:2174223-2181005	607	731028

Table S2. Homological analysis of amino acid sequences of PtNAD-ME proteins in *Populus trichocarpa*.

	PtNAD-ME1	PtNAD-ME2	PtNAD-ME3	PtNAD-ME4
PtNAD-ME1	100	96	66	65
PtNAD-ME2		100	66	65
PtNAD-ME3			100	92
PtNAD-ME4				100

Table S3. The sequences of primers for amplifying the CDS of *PtNAD-MEs*.

Gene	Primers for CDS (5'-3')
<i>PtNAD-ME1</i>	CACCATGCCGAATTTTCCAATCAGA; TCAATCTTCTTATAGACCAGTGTG
<i>PtNAD-ME2</i>	CACCATGTCAAATTTCCAATCAGAT; TCAATCCCTCTTATAGACCAATGTG
<i>PtNAD-ME3</i>	CACCATGTGGAGATTAGCGCGATGTG; CTATTCTCATGAACAAGAGGGCTGT
<i>PtNAD-ME4</i>	CACCATGTGGAGGGTAGCGCGATTG; TTATTCTCATGAACAAGAGAGCTGTA

Table S4. The sequences of primers for real-time PCR of *PtNAD-MEs*.

Gene	Primers for semi-RT-PCR (5'-3')
<i>PtNAD-ME1</i>	AGCTCCGAAAATCAGCCAG; CTCAGATTTCAGGCAGTCAG
<i>PtNAD-ME2</i>	AGGATACCGTCAAATGGATGCT; TTGACAACATGTTGATCCCCA
<i>PtNAD-ME3</i>	CAGAAGGACATGGTGAGGCA; ACTCGAGCCCTATTCTCATGA
<i>PtNAD-ME4</i>	TGTGGCATATGTCATGCAGA; AATATCGAGGCTGGATGCAG
<i>PtActin2</i>	GTTTCCAGGAATAGCTGATCG; CCTCCGATCCAAACACTGTA

Table S5. Primers for amplifying the CDS for each mature PtNAD-ME proteins without signal peptides.

Protein	Peptide number	Primer sequences (5'-3')
PtNAD-ME1	592	<u>GGATCC</u> ACTACATTGGAGGGTCACCG (<i>BamH I</i> site underlined) <u>CTCGAG</u> TCAATCTTCTTATAGACCAGT (<i>Xho I</i> site underlined)
PtNAD-ME2	592	<u>GGATCC</u> ACTACATTGGAGGGCCACCG (<i>BamH I</i> site underlined) <u>CTCGAG</u> TCAATCCCTCTTATAGACCAA (<i>Xho I</i> site underlined)
PtNAD-ME3	586	<u>GGATCC</u> ACTGATATTCTCATGATCC (<i>BamH I</i> site underlined) <u>CTCGAG</u> CCTATTCTCATGAACAAGAG (<i>Xho I</i> site underlined)
PtNAD-ME4	586	<u>GGATCC</u> GATATCCTCATGATCCTTG (<i>BamH I</i> site underlined) <u>CTCGAG</u> TTATTCTCATGAACAAGAGA (<i>Xho I</i> site underlined)

Figure S1. Exon/intron structures of the PtNAD-ME genes. Grayer boxes represent exons and black lines represent introns.

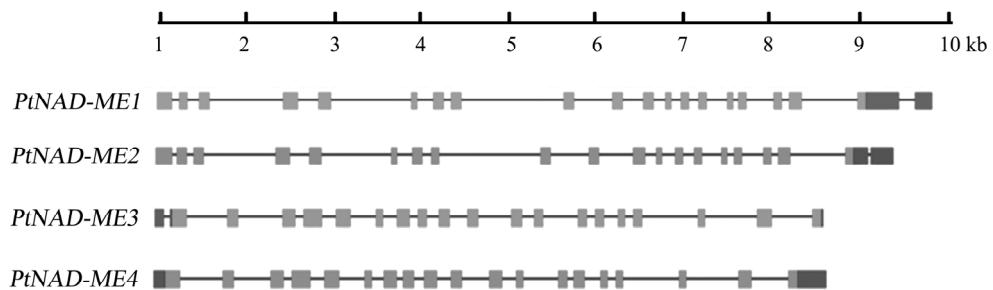


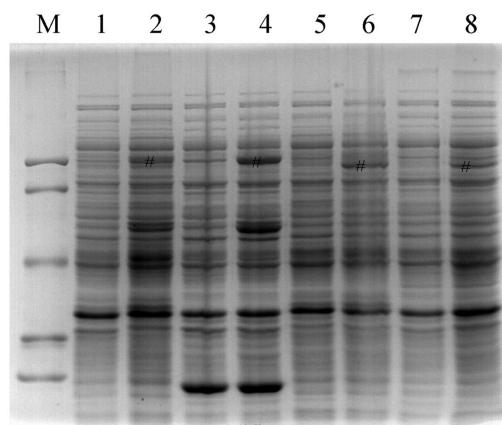
Figure S2. Multiple sequence alignment of full-length amino acid sequences of *Populus* NAD-ME proteins. Sequences were aligned using ClustalW2 program (<http://www.ebi.ac.uk/Tools/msa/clustalw2>). Consensus sequence is marked with *.

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P tNAD-ME3      ---MWRLARCASSNLRSRLRR-----RFFSTAAIPAPCIIHKRGTDILHDPWFN 46
P tNAD-ME4      ---MWRVARFAASNVRSSQR-----RFFSAAAIPGACIVHKRGADIHKDPWFN 46
P tNAD-ME1      MPNFSNQIRASSSLIKRLQQRMTPAALMQATRSFTTLEGHRPTIVHKRSLDILHDPWFN 60
P tNAD-ME2      MSNFSNQIRASSSLIKRLKQRMTNPAAALMQATRHFTTLEGHRPTIVHKRSLDILHDPWFN 60
                : . * ::* : :* * * : . *:***. *****
P tNAD-ME3      KDTGFPLTERDRLGLRGLLPPRVISFEQQYDRFMEYSRSLEKNTQGQPYSVVSIAKWRIL 106
P tNAD-ME4      KDTGFPLTERDRLGLRGLLPPRVISFEQQYDRFMEYSRSLEKNTQGQPYSVVSIAKWRIL 106
P tNAD-ME1      KGTAFSMTERDRLDIKGLLPPNMSSSEQIQRFMVDLKRLEVQARDGSPDPNALAKWRIL 120
P tNAD-ME2      KGTAFSMTERDRLDLRGLLPPNVMTSEQIQRFAADLKRLEVQARDGSPDPYALAKWRIL 120
                *.*. :*****. :*****. *: *** :** . : ** : :. * . :*****
P tNAD-ME3      NRLHDRNETLYYRVLIDNIKDFAPIYTPTVGLVCQNYSGLFRRPRGMYFSAKDKGEMMS 166
P tNAD-ME4      NRLHDRNETLYYRVLIDNIKDFAPIYTPTVGLVCQNYSGLFRRPRGMYFSAKDKGEMMS 166
P tNAD-ME1      NRLHDRNETMYFKVLIANIEEYAPIVYTPTVGLACQNYSGLFRRPRGMYFSAE DRGEMMS 180
P tNAD-ME2      NRLHDRNETMYQVQLIANIEEYAPIVYTPTVGLVCQNYSGLFRRPRGMYFSAE DRGEMMS 180
                *****: *: *** :** : :*****. *****:*****:*****:*****
P tNAD-ME3      MLYNWPAAQVDMILTDGSRLILGLGDLGVQIGIPIGKLDMYVAAAGINPQKILPVMLDV 226
P tNAD-ME4      MLYNWPAGQVDMILTDGSRLILGLGDLGVQIGIPIGKLDMYVAAAGINPQRIIPIMLDV 226
P tNAD-ME1      MVYNWPAAEQVDMIVVTDGSRLILGLGDLGVQIGIAIGKLDMYVAAAGINPQRVLPMIDV 240
P tNAD-ME2      MVYNWPAAEQVDMIVVTDGSRLILGLGDLGVQIGIAIGKLDMYVAAAGINPQRVLPMIDV 240
                :****. :*****:*****:*****. *****:*****:*****:*****
P tNAD-ME3      GTNNQKLLLEDPLYLGLRQPRLEGEEYLSIVDEFMEAHTRWPKAIVQFEDFQMKWAFETL 286
P tNAD-ME4      GTNNQKLLLEDPLYLGLRQPRLEGEEYLSIVDEFMEAHTRWPKAIVQFEDFQMKWAFETL 286
P tNAD-ME1      GTNNEKLLKDPLYLGLQEHRLDGDEYIAVIDEFMEAFTRWPHIVIVQFEDFQSKWAFKLL 300
P tNAD-ME2      GTNNEKLLKDPLYLGLQENRLDGDEYIAVIDEFMEAFTRWPHIVIVQFEDFQSKWAFKLL 300
                ****:*****:*****: *: : : :*****. ***:***** ****: *
P tNAD-ME3      QRYRKRCFMNDDIQGTAGVALAGLLGTVRAQGPLSDFVNQKI VVVGAGSAGLGVLNMA 346
P tNAD-ME4      QRYRKRCFMNDDVQGTAGVALAGLLGTVRAQGRPLSDFVNQKI VVVGAGSAGLGVL TMA 346
P tNAD-ME1      QRYRNAYRMFDDVQGTAGVAIAGLLGAVRAQGRPMIDFPKQKIVVAGAGSAGIGVLNA 360
P tNAD-ME2      QRYRNAYRMFDDVQGTAGVAIAGLLGAVRAQGRPMIDFPKQKIVVAGAGSAGIGVLNA 360
                ****: : ****:*****:*****:****: *: ** :****, *****: ***
P tNAD-ME3      IQALSRMSGNNEMAANKNCYLLDKGLITKERNDIDPAAPFAKDLKDVE--EGLREG 401
P tNAD-ME4      IQALSRMSGNNEMAANKNCYLLDKGLITKERNDIDPAAPFAKDLKDVE--EGLREG 401
P tNAD-ME1      RKTMARMLGNNESAFESAGRQFWVDAKGLITTEERENIDLEALPFARKVEEASRQGLREG 420
P tNAD-ME2      RKTMARMLGNNESAFESAGRQFWVDAKGLITTEERENIDPEALPFARKVKEASRQGLREG 420
                : : :*** *** : * . : : :* . ****:***: * * ***: . : :*****
P tNAD-ME3      ASPFEVVKKLKPHVLLGLSGVGGVFNEEVLKAMRESDSTKPAIFAMSNPTMNAECTAADA 461
P tNAD-ME4      ASLLEVVKKLKPHVLLGLSGVGGI FNEQVLUKAMRESDSTKPAIFMSMSNPTMNAECNAADA 461
P tNAD-ME1      ASLAEVVREVVKPDVLLGLSAVGLFLSKEVLEALKGSTSTRPAIFAMSNPTKNAECTPEEA 480
P tNAD-ME2      ASLAEVVREVVKPDVLLGLSAVGLFLSNEVLEALKGSTSTRPAIFAMSNPTKNAECTPEEA 480
                ** ***: :***. *****: *: : : :* : * . :****:***** ***. : *
P tNAD-ME3      FKYAGPNIIFGSGSPFEDVLDNGKVGHVNQANMNYLFPGI GLGTLLSGAHI ITDGMQLQA 521
P tNAD-ME4      FKHAGPNIVFASGSPFENVLDNGKVGHVNQANMNYLFPGI GLGTLLSGAHV ITDGMQLQA 521
P tNAD-ME1      FSIVGDNII FASGSPFKDWDLNGHIGHCNQGNMNYLFPGI GLGTLLSGSRI ISDGMLQA 540
P tNAD-ME2      *. . * **: . *****:*****: ** * . *****:*****: : :*****: ****
P tNAD-ME3      AAECLASYMTDEEQNGILYPSIDSIRHITAEVGAVALRAAVEEDLAEGHGEAGPRELKH 581
P tNAD-ME4      AAECLASYMTDEEQKGILYPSIDSIRHITAEVGAVALRAAVEEDLAEGHGDVGPRELKH 581
P tNAD-ME1      AAECLAAYMTEEEVLKGIIYPSTSRI RDITKEVAAAVVKEAIEEDLAEGYREM DARELRK 600
P tNAD-ME2      AAECLAEMYMAEEEV LNGIIYPSTSRI RDITKEVAAAVVKEAIKEDLAEGYREM DARELRK 600
                *****: *: : : :***: *** . **. ** . ***: : :*****: : . ***: :
P tNAD-ME3      MSKAETVAYVSRNMWFPVYSPLVHEK- 607
P tNAD-ME4      MSKEETVAYVMQNMWFPVYSSLVHEK- 607
P tNAD-ME1      LSQEEIEEYVKNNMWSPDYPTLVYKKD 627

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Figure S3. SDS-PAGE analysis of the four recombinant *Populus* NAD-MEs without mitochondrial signal peptides expressed in *E. coli* BL21 cells. M, marker (91, 67, 43, 31, and 20 kDa); 1-8, 0 and 2h IPTG induction for cells with pGEX-6P-3-PtNAD-ME1, pGEX-6P-3-PtNAD-ME2, pGEX-6P-3-PtNAD-ME3, and pGEX-6P-3-PtNAD-ME4, respectively. Pound key indicate each GST-PtNAD-ME proteins.



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