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Supplementary Material

Possible involvement of phosphoenolpyruvate carboxylase and NAD-malic enzyme in response to drought stress. A case study: a succulent nature of the C_4-NAD-ME type desert plant, *Salsola lanata* (Chenopodiaceae)

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Table S1. Primers used for PCR

Abbreviations: *18S rRNA*, *ribosomal RNA 18S*; cyt, cytosolic; *EF1-α*, *elongation factor-α*; *GAPDH*, *glyceraldehyde 3-phosphate dehydrogenase*; NAD-ME, NAD-malic enzyme; PEPC, phosphoenolpyruvate carboxylase

Gene	Sequences (5’ to 3’)
Target genes	
PEPC	Forward: GGKGCATACATYATCTCCATG
	Reverse: GGTGTCCTCAAGGCCAGGAG
α-NAD-ME	Forward: TCCTACWGTWGGTCTTGTTTGCC
	Reverse: AATYTTTTGTTTAGGGAAGTCAATCA
Candidate reference genes	
β-actin	Forward: ACATCWGCCGAACGGGAAA
	Reverse: CAACCTTGATCTTCATGCTGCTAG
EF1-α	Forward: TCAYRTCAGTTTGGTGGTTATTGGA
	Reverse: CCAAGRGTGAAGGWAAGAAGRGC
18S rRNA	Forward: GCTCGTAGTTGGACCTTGGG
	Reverse: AAGGGCAGGGASGTAGTCAAC
α-tubulin	Forward: TSGACATTGAGCGWCCCACWT
	Reverse: CACAGCAGCRTTSACATCCTT
GAPDH	Forward: CAGAGRGAYGATRTYGAGCTTGT
	Reverse: GARCTGTAWCCCCATTCGTTGTCAT

Table S2. Primers used for gene expression of *Salsola lanata* under drought for RT-qPCR

Abbreviations: *18S rRNA*, *ribosomal RNA 18S*; cyt, cytosolic; *EF1-α*, *elongation factor-α*; *GAPDH*, *glyceraldehyde 3-phosphate dehydrogenase*; NAD-ME, NAD-malic enzyme; NADP-ME, NADP-malic enzyme; PEPC, phosphoenolpyruvate carboxylase

Gene	Sequences (5' to 3')	Annealing temperature (°C)
Target genes		
PEPC	Forward: GTTATGGCGAAACCAAAGACC	Forward: 59.0
	Reverse: TGCCTGGCAAACATTTAGGAT	Reverse: 59.9
α-NAD-ME	Forward: AGAAGGCCACGAGGAATGTA	Forward: 57.2
	Reverse: CTAGGTCTCCAAGTCCCAAAAT	Reverse: 57.1
Candidate reference genes		
β-actin	Forward: TCCACGAAACAACCTACAACTC	Forward: 57.0
	Reverse: CAGCAATACCGGGGAACAT	Reverse: 58.0
EF1-α	Forward: TCAGTTTGGTGGTTATTGGACA	Forward: 58.0
	Reverse: ACCTCTTGTTCATCTCAGCAGC	Reverse: 58.6
18S rRNA	Forward: GGGCATTCGTATTTCATAGTCA	Forward: 57.3
	Reverse: CGGCATCGTTTATGGTTGA	Reverse: 57.5
α-tubulin	Forward: CCATACCCCAGGATTCACTTC	Forward: 58.4
	Reverse: CACACTTTGCCATCATAGACGA	Reverse: 58.7
GAPDH	Forward: AACGAGCACGAATACAAGTCAG	Forward: 57.4
	Reverse: AGCCCCTCAAGAATACCGA	Reverse: 57.0

Table S3. The expression stability value (SV) of candidate reference genes in all samples

Abbreviations: *18S rRNA*, *ribosomal RNA 18S*; *EF1-α*, *elongation factor-α*; *GAPDH*, *glyceraldehyde 3-phosphate dehydrogenase*

Software programs	Five candidate references				
	β-actin	*α-tubulin*	*GAPDH*	*EF1-α*	*18S rRNA*
NormFinder	0.004	0.009	0.013	0.047	0.049
geNorm	0.041	0.044	0.045	0.073	0.076

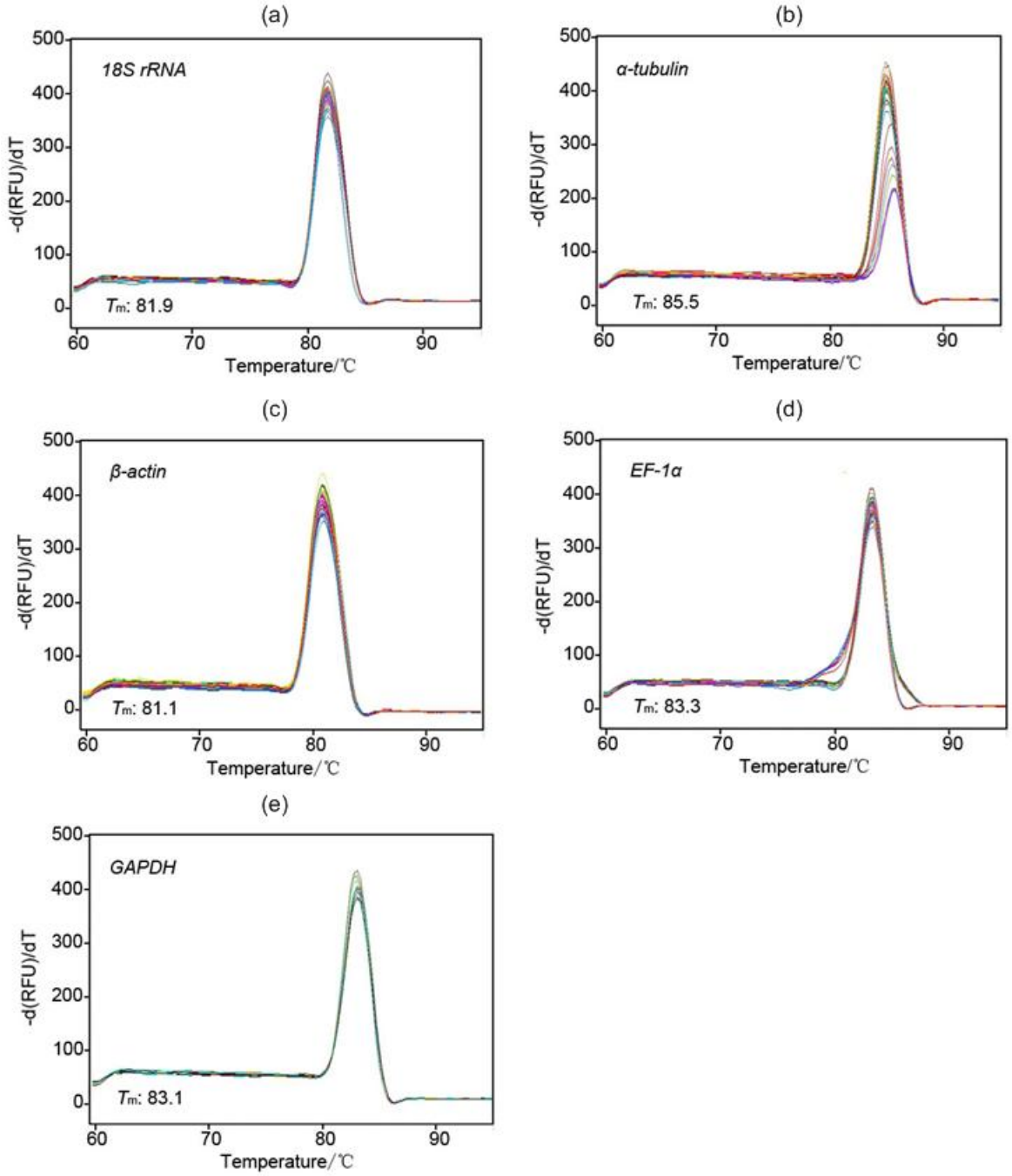

Fig. S1. RT-qPCR melting curves of five candidate reference genes of *Salsola lanata*.

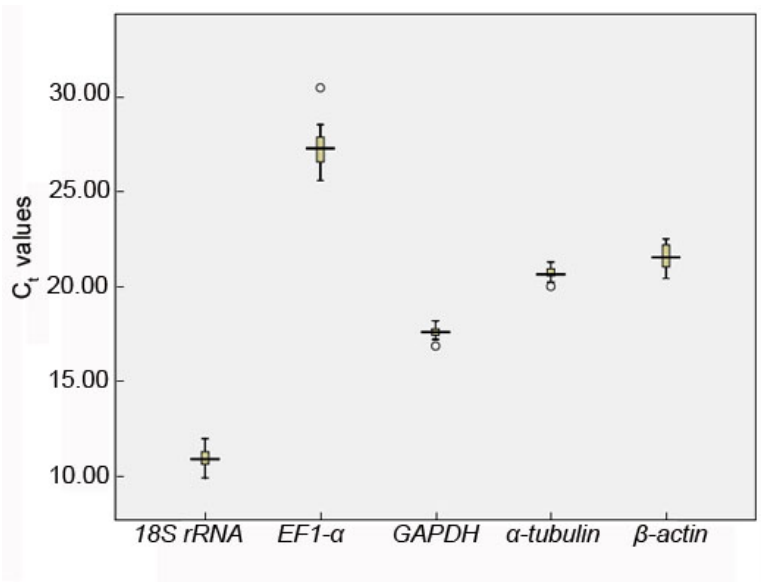

Fig. S2. Distribution of C_t values of five candidate reference genes across all samples. The Box-plot contains the mean, interquartile range, non-outlier range, and outlier.