Table S1: Forward (Fwd) and reverse (Rev) primers used to determine gene expression levels via quantitative real-time PCR. The Alfalfa Gene Index and Expression Atlas Database was used to obtain the coding sequences for the genes of interest which are given as contig numbers.

Gene	Contig ID	Sequence (5′→ 3′)	Amplicon length (bp)	Efficiency (%)
Polyamine synthesis				
arginine decarboxylase	46745	Fwd: GCTTGAATCTCTTCACGCC	114	109
		Rev: TCCTCCACAACAAACCTATCC		
diamine oxidase	14769	Fwd: CTGGTGGTCAGAATGATTGC	89	102
		Rev: ACCCCAACTTTTATGCTGC		
glutamate decarboxylase	103359	Fwd: ATGGATGAATACCCCGTCAC	114	107
		Rev: AACAGTGCCAACACCAACTG		
SAM decarboxylase	9152	Fwd: CAAGCAAGTCGTTTGAGCAG	94	101
		Rev: ACAGAACCACCCATTCCAAG		
spermidine synthase	5341	Fwd: ACCGACATTCACACCAGAC	137	107
		Rev: TCTCCTTCTCCAAACCATTCC		
spermine synthase	92526	Fwd: TCTTTTATGTCATCCAAACCCC	134	108
		Rev: AATCCACCACCTCCTTGTC		
Flavonoide synthesis				
chalcone isomerase	60809	Fwd: CAAGGCAGTTTCATCAGCAG	113	108
		Rev: AAGCACCCTCGTTCAACAAC		
chalcone reductase	59796	Fwd: TCCTGCTCTTCAAAAATCTCTC	150	108
		Rev: TCCCAAACACCTTTCACATC		
chalcone synthase	99463	Fwd: ACCTTCATTGGATGCTAGGC	132	108
		Rev: GTGGTGCAAACGATCAAGTG		
isoflavone synthase	10252	Fwd: TGCTATGGTTCCCTTTGGAC	112	102
		Rev: GATTTCTCGGCTCCTCAATG		
Glutathione synthesis and redox cycle				
homoglutathione synthetase	Cui et al. 2012	Fwd: GGCACCAAAAAGATTCAACA		122
		Rev: AACTCCACAACCCTGCAAAG		
γ-glutamylcysteine synthetase	Cui et al. 2012	Fwd: CATGGAAAGTGGGAGCAATC		102
		Rev: AGCAAGAAGCCGAATACACC		
glutathione reductase 1	Cui et al. 2012	Fwd: AGGACGGTGAACCTGATTTG		99
		Rev: GAATCTAGCAGCACGAACAC		

glutathione reductase 2	Cui et al. 2012	Fwd: TTCCGTTCTCCACAATCTCAT Rev: TTCACTGTCATATCTCCATCCAA	90
monodehydroascorbate reductase	Cui et al. 2012	Fwd: TCAAAGGGCAGGTTGAAGAG Rev: AAGGGAACGTAGCAACATCG	102
glutathione synthetase 2	10444	Fwd: AACGATGGCAGAAGTTGACC Rev: AGCCAGCCCTGAAGTAAATG	102
Reference genes			
cyclophilin	Guerriero et al., 2014	Fwd: CAAACTTTCCTGACGAGTCACC Rev: ACGGTCAGCAATTGCCATTG	104
eIF4A	Guerriero et al., 2014	Fwd: TGCTAAGTTGCCTGAAACCG Rev: TGCCCATGTTTTCACCTTGC	96

Table S2: Quantitative real-time PCR parameters according to the Minimum Information for publication of Quantitative real-time PCR Experiments (MIQE) guidelines derived from Bustin *et al*. (2009). All procedures were performed according to the manufacturer's protocols.

Sample/Template	
Source	Stems of *Medicago sativa* plants cultivated in soil
Method of preservation	Liquid nitrogen
Storage time	12 month at -80 °C
Handling	Frozen
Extraction method	Phenol-free Total RNA isolation: RNAqueous™ Kit (Life Technologies)
RNA: DNA-free	TURBO DNA-free™ Kit (Life Technologies) Design of intron-spanning primers whenever possible
Concentration	NanoDrop®: ND-1000 Spectrophotometer (Thermo Fischer Scientific)
Assay optimisation and validation	
Accession number	Table S1
Amplicon details	Amplicon size: Table S1
Primer sequences	Table S1
In silico	Primer design with the Primer3Plus online tool (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and primer analysis with OligoAnalyzer 3.1 (https://eu.idtdna.com/calc/analyzer)
Empirical	A primer concentration of 100 nM was used Annealing temperature: 60°C
Priming conditions	Combination of oligodT-primers and random hexamers
PCR efficiency	Dilution series (slope, y-intercept and r^2; Table S1)
Linear dynamic range	Samples are situated within the range of the efficiency curve
Reverse transcription - PCR	
Protocols	As stated in the materials and methods section
Reagents	As stated in the materials and methods section
No template control (NTC)	Cq and dissociation curve verification
Data analysis	

Specialist software	ViiA7 Real-Time PCR system (Applied Biosystems)
	qBase^{PLUS} software v2.5 (Biogazelle)
Statistical justification	Five biological replicates
	Relative expression of each gene was calculated as an average after elimination of outliers
	Log transformation of the data
	Significance of changes in gene expression was determined automatically qBase^{PLUS} software v2.5 (Biogazelle)
Normalisation	Two reference genes were selected as described in the materials and methods section