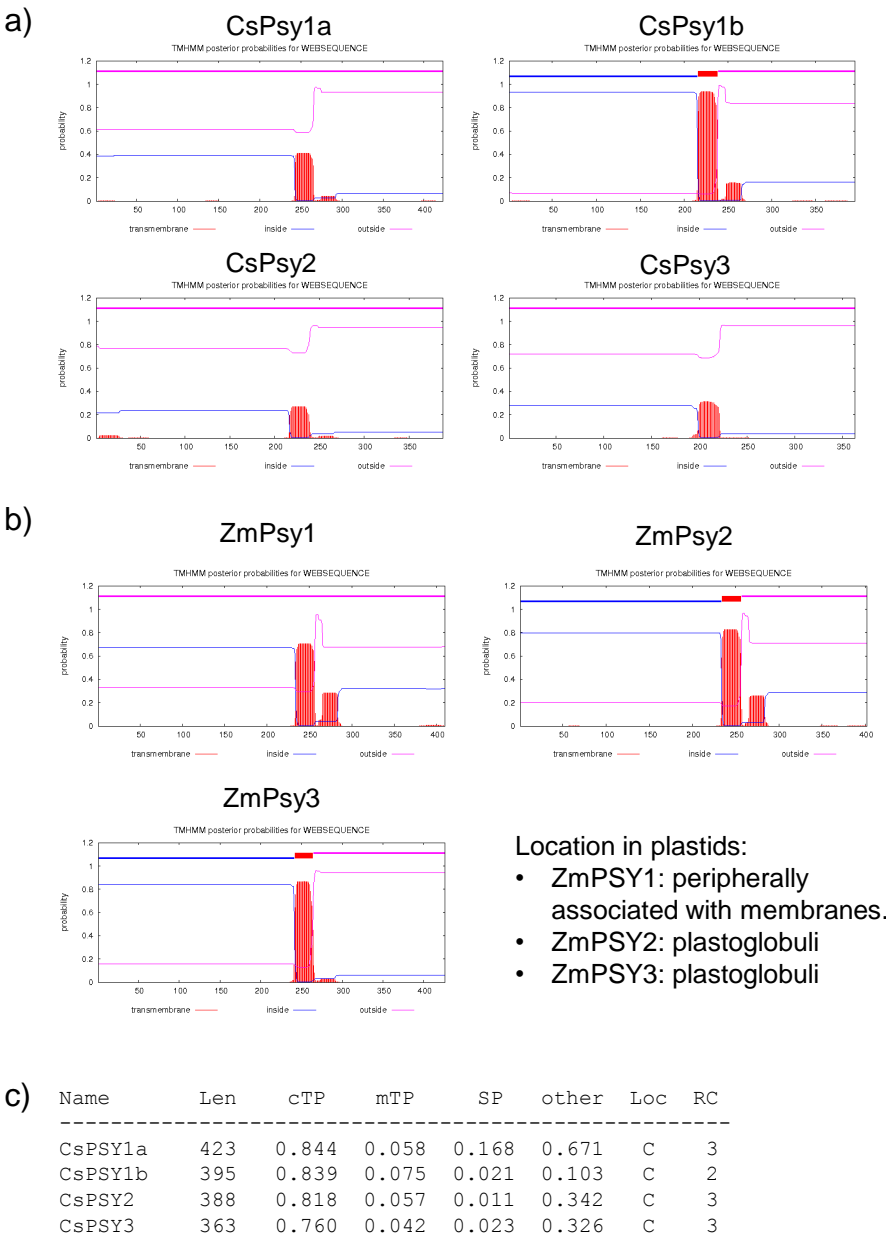
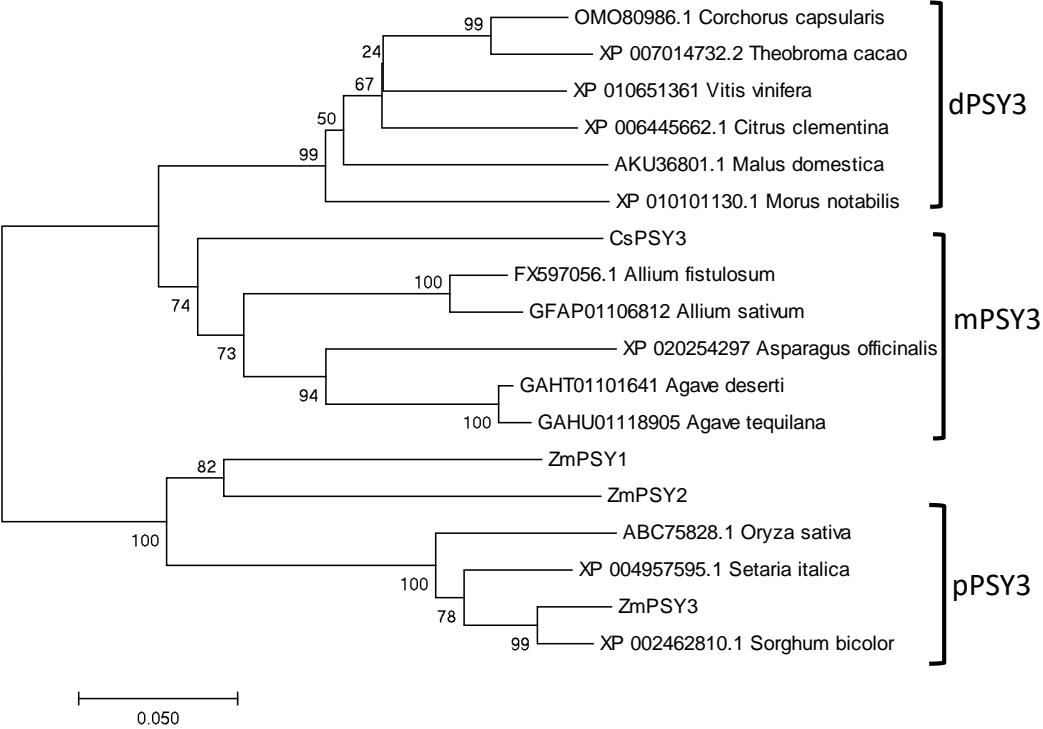


Supplementary Figure S1



**Supplementary Figure S1.** (a) Putative hydrophobic profiles of different CsPSY enzymes, evaluated by the TMHMM Server v. 2.0. (b) Putative hydrophobic profiles of different ZmsPSY enzymes, evaluated by the TMHMM Server v. 2.0 and their location in plastids according to Shumskaya et al., 2012. (c) Prediction of the subcellular location of CsPSY proteins using the TargetP 1.1 Server.

Supplementary Figure S2



**Supplementary Figure S2.** Phylogenetic tree (neighbor-joining) of PSY3 enzymes from monocots (excluding the Poaceae sequences: mPSY3), Poaceae sequences (pPSY3) and dicots (dPSY3). The length of branch expresses evolutionary distance, with its scale being 0.05.

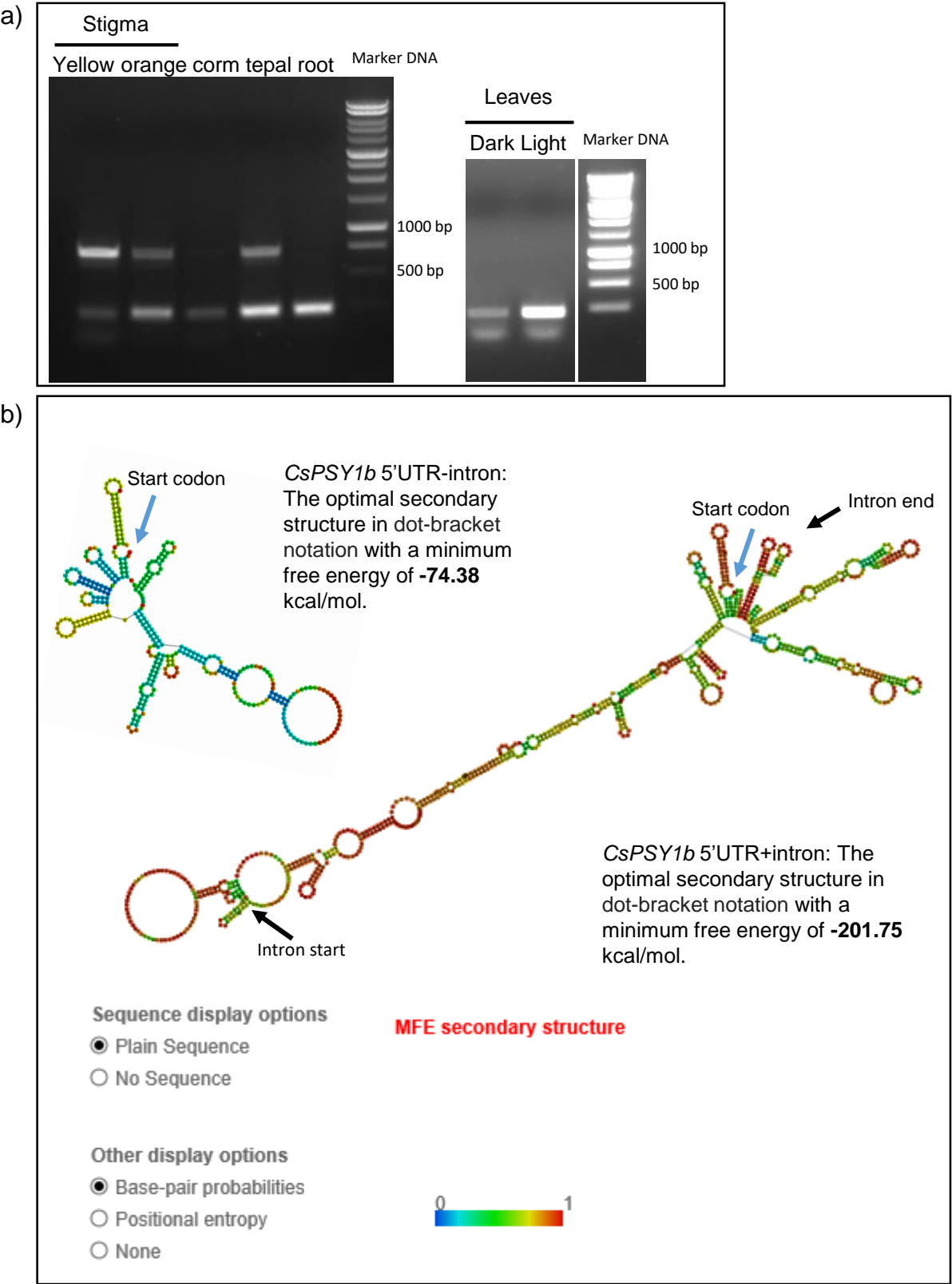
Supplementary Figure S3

*CsPSY1b* 5'UTR

AGAAATAATAGTAATAAAAAATTTGCATTATTTTATCTTCTAAGAAAATGATTGGTGATTTTTTTTTTCTC  
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GGTATATAATGGTGTGGTAGTGGTTGATAGTCATTCATCACATGCTATTTGGAAACACAAAGATAAGAGA  
TACCCAATTCCACCACTTAACTTGCCCCTCTCTTCTTCTTCTTCTTCTTCTTCTTCTTCAAAGTTCATTC  
ACTTCTTTTGGGGGCATCTTGTCAATTATTGAATACTGAAGGTAACATACTATTCATATCATATTCCTTTT  
TGGTATCTTAGCATTGTTCTTTTTTAATGTATGTCCATTTTCATCCAAGTGGCTCTACTTTTTCTCTTGA  
ATGCTAATAACACCATACTATTCCTAGGATAATTTTGGCATGTGGGTCTTGGTTGTTATGATTATTGTCT  
AGCTTTTGTTGGTATATCTCCACTTCTTCTCTTTTTTGTTTTTTGTAAGAGTAGAAAAAAGGAACCTCC  
TTTTTTCTCTCCCTTCATCATGTCAATTGTTGATTGGATACTAAAGATGACACTTTTCCTCTTTTCTTGGT  
ATGGATTTACCAAGTCATCTAAGTTTTTCAATATTGAGCAATGAATCTGAATATCTGTTATAAATACCC  
ATCAGAAGTTCCTCTCCTTTGTGTTTCAATCATGTCACTGTTGATTGGATACTGAAAAGTAACATCTTGA  
AGAATTTTAGCATATGGGTTTGTGATAGTTTATGATTACCTTGTGATTCATGTAGTTAATTTACTTACAA  
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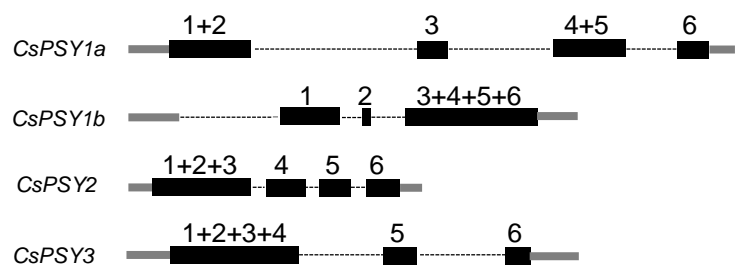
**Supplementary Figure S3.** *CsPSY1b* 5'UTRs with the intronic sequence in white background, and the start codón in red.

Supplementary Figure S4



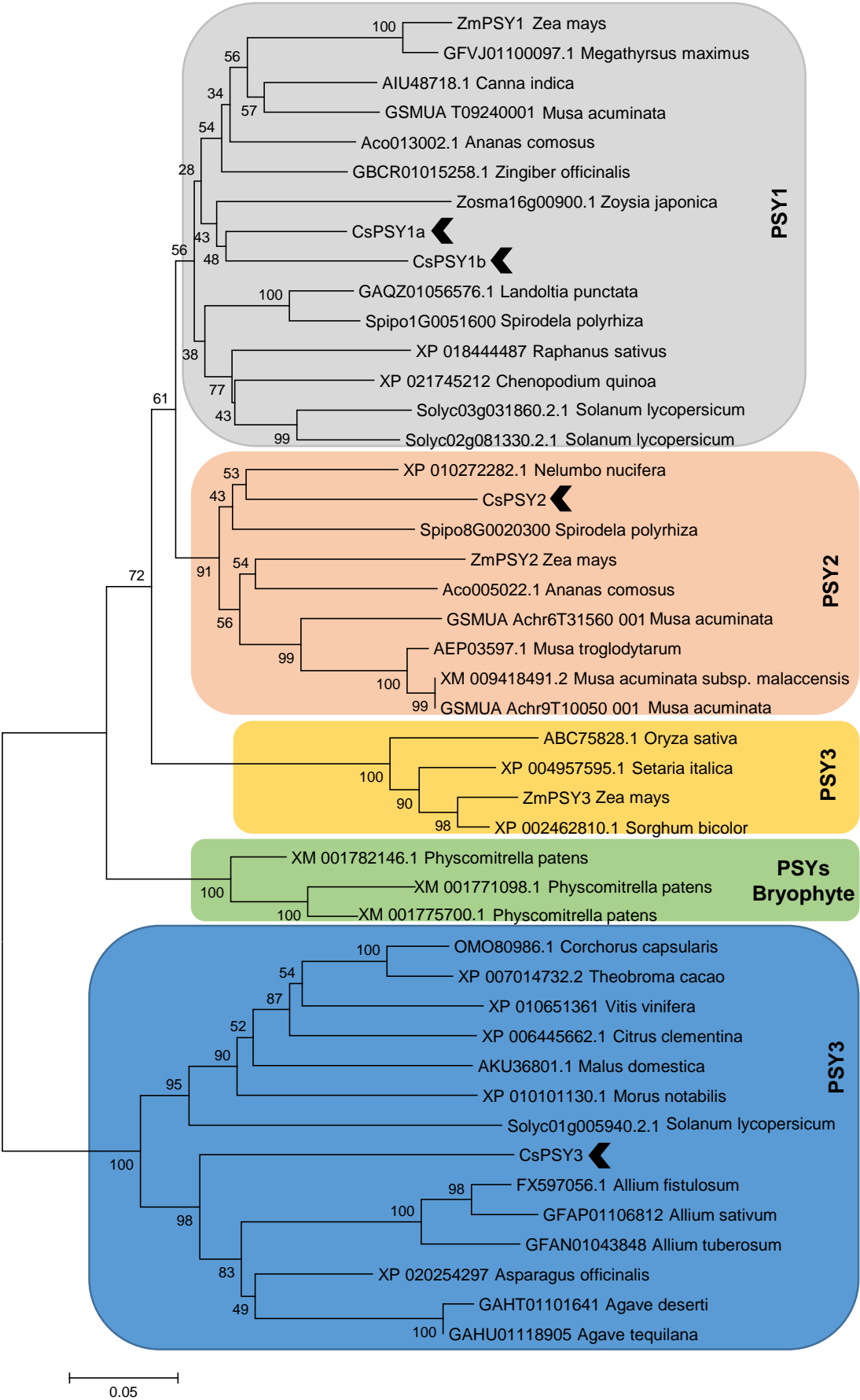
**Supplementary Figure S4.** Selective expression of alternative *CsPSY1b* variants. (a) Agarose gel showing the results of RT-PCR over *CsPSY1a* 5'UTR. (b) Structures of *PSY* 5'UTRs with or without the intron sequence performed with RNAalifold .

Supplementary Figure S5



**Supplementary Figure S5.** Exon/intron structure of genomic *CsPSY* genes. Identified exons are shown as solid black blocks. Introns are shown as dotted lines and untranslated regions are shown as grey lines.

Supplementary Figure S6



**Supplementary Figure S6.** Phylogenetic tree (neighbor-joining) of PSY enzymes. Indicated by a black arrow are CsPSY enzymes isolated in this study. Numbers associated with the branches are the neighbor-joining bootstrap values. The length of branch expresses evolutionary distance, with its scale being 0.05.

Supplemental Table S1. Oligonucleotide sequences used in this study.

	Forward 5'-3'	Reverse 5'-3'
For cDNAS isolation	1-GARTAYGCIAARACITTYTAYYT 2-ATHTGGGCIATNTAYGTITGGTG	TCRTCYTGGIGGIAGRTADATYCT
For RACE-PCR		
CsPSY-1a	GTGTTCCGGTGATGGGTATCG	CTTGAATGGCTGAATGTCAAC
CsPSY-1b	TGCACCAGACTCTCAAGCCAC	GTCCATCTACAAGCTCATCTGT
CsPSY-2	TCAGCTTACCAACATACTAAGA	CATCCACAAGTTCATCGGTTC
CsPSY-3	AGGCATGCAACTACCAACATC	TCTTGCCATCGATCAAGGACA
For full-length cDNA and gDNA isolation		
CsPSY-1a	ATGGCAATCGCATTACTTCGAGT	TCATCCTTTCAAGAGGCTCGACT
CsPSY-1b	ATGGCAACTCAAATGATGAGGGT	CTAGGCATTACCAGGTTGGCTAC
CsPSY-2	ATGTCTGGTTCGTGTGTTTTGTTG	TCAGACTTTTGAAGGACCAAATG
CsPSY-3	ATGAGTACCTCCATTTTACCAAAT	AGCTTTAGCAAATCCCCAAGGCA
For expression analyses		
CsPSY-1a	TTCGCCCATAGAATTTCCAA	TAAACCCTCTGCTCCGAAGA
CsPSY-1b	GTTGTCTCACCGGTGGAAGT	TCACCAATTCGAGGTTGACA
CsPSY-2	TTTCGGTCACCACCGATAAG	TCATGAGCAATGTTCCCAAG
CsPSY-3	CACTATCCAGCTTCCCTCCA	CCACGACACCTCCTATAGCC
For activity assays		
CsPSY-1a	CGCCCTTGGCGAATTCTGAAGGAGACCTTAG AGGA	TACCCTCGAGGAATTCTCATCCTTTCAAG AGGCTC
CsPSY-1b	CGCCCTTGGCGAATTCTGCAACCCGGTGAA AGAT	TACCCTCGAGGAATTCTCATTACCAGGTT GGCTAC
CsPSY-2	CGCCCTTGGCGAATTCCATCACTTCACTGG AAGG	TACCCTCGAGGAATTCTCAGACTTTTGAA GGACCA
CsPSY-3	CGCCCTTGGCGAATTCTGTGAACGAGGTCGT GGTA	TACCCTCGAGGAATTCTCAAGCAAATCCC CAAGGCA
For GFP fusion		
CsPSY-1a	GGACTCTTGACCATGGATGGCAATCGCATT ACTTCGAGT	GTCAGATCTACCATGGTCCTTTCAAGAGG CTCGACTGCC
CsPSY-1b	GGACTCTTGACCATGGATGGCAACTCAAAT GATGAGGGT	GTCAGATCTACCATGCGGGCATTACCTTG GCTACCTTGC
CsPSY-2	GGACTCTTGACCATGATGTCTGGTTCTGTT GTTTTGTTGATTTC	GTCAGATCTACCATGCCGACTTTTGAAGG ACCAGCAAGAGC
CsPSY-3	GGACTCTTGACCATGATGAGTACCTCCATT TTACCAAATCTCTCTATG	GTCAGATCTACCATGAGCTTTAGCAAATC CCCAAGGC
For 5'UTR CsPSY1b analyses	AGTGGTTGATAGTCATTCATC	GACAACCCTCATCATTTGAGT

Supplemental Table S2. Characteristics of the PSY proteins from saffron.

Name	Accession number	cDNA size (bp)	Protein size (aa)	Mw (KDa)	PI
CsPSY1a	MH124237	1272	423	47.79	8.66
CsPSY1b	MH124238	1187	395	44.24	8.86
CsPSY2	MH124239	1209	388	43.97	9.92
CsPSY3	MH124240	1098	366	42.53	6.28



Supplemental Table S3. Characteristics of the saffron PSY genomic clones

	Intron number	Intron size	5'UTR	3'UTR
CsPSY1a	3	958/619/316	307	149
CsPSY1b	3	536 (5'UTR)/143/256	890	235
CsPSY2	3	73/90/89	167/107	103
CsPSY3	2	524/539	264	293

Supplemental Table S4. Mass pair (m/z) and compound-dependent parameters of the standards.  
† C.E.: Collision Energy

Compound name	t <sub>R</sub> (min)	Quantifier ion (m/z)			Qualifier ion (m/z)		
		Q1 Precursor ion	Q3 Product ion	C.E. <sup>†</sup> (eV)	Q1 Precursor ion	Q3 Product ion	C.E. <sup>†</sup> (eV)
(±)-GR24 (IS)	3.47	321	224	11	-	-	-
Orobanchol	3.67	347	233	12	347	329	6