

**Table S1.** Sequence homology of SQS proteins in the indicated species.

Gene	Homology							
<b>Yeast</b>	100%							
<b>Human</b>	47.5% 100%							
<b>Chlamy</b>	39.2% 46.5% 100%							
<b>OsSQS1</b>	41.7%	48.1%	54.8%	100%				
<b>AtSQS1</b>	41.7%	45.9%	54.8%	72.6%	100%			
<b>GmSQS</b>	43.0%	48.1%	56.7%	79.0%	81.8%	100%		
<b>MtSQS</b>	42.0%	48.4%	56.7%	77.1%	79.0%	91.4%	100%	
<b>MsSQS</b>	41.4%	48.7%	57.0%	76.8%	79.0%	92.0%	97.8%	100%

**Table S2.** Sequence of primers used in this study.

Primer name	Sequence	Application
MsSQS-f	5'-ATGGGAAGywTrArwrCGATTTG-3'	ORF
MsSQS-r	5'-TTAkwywmTTryrwyGATTkGCwkwGAGA -3'	amplification
SQS-PE-f	5'-ATGGGAAGTATAAAAGCGATTTG-3'	Prokaryotic
SQS-PE-r	5'-GTTATTGTAACGGTTGGCAGAGAG-3'	expression
SQS-PE-ΔC30-r	5'-CCTCAAAACGTAAGATTCCCTTTGG-3'	
SQS-pA7-f	5'- <u>CCCTCGAG</u> ATGGGAAGTATAAAAGCGATTTGA-3'	subcellular
SQS-pA7-r	5'- <u>GCGTCGAC</u> GTATTGTAACGGTTGGCAGAGAGA-3'	localization
SQS-QPCR-f	5'-CTTCGGTCTTGTATTCAACGGTGTC-3'	PCR/qRT-PCR
SQS-QPCR-r	5'-CTTGTATCATCCTCAACGGTGTC-3'	
Actin-f	5'-CAAAAGATGGCAGATGCTGAGGAT-3'	house-keeping
Actin-r	5'-CATGACACCAGTATGACGAGGTCG-3'	gene
SQS-f	5'- <u>GCTCTAGA</u> ATGGGAAGTATAAAAGCGATTTGA-3'	overexpression
SQS-r	5'- <u>CGGGATCCT</u> TAGTTATTGTAACGGTTGGCAGAG-3'	
35S-f	5'-ACTATCCTTCGCAAGACCCTTCCTC-3'	Identification of transgenic lines
SQS-r	5'- <u>CGGGATCCT</u> TAGTTATTGTAACGGTTGGCAGAG-3'	
gSQS-f1	5'-CTGAAAATCGCGGCGAGAAA-3'	
gSQS-r1	5'-AGCTTGATAAACCCAGCCCA-3'	
gSQS-f2	5'-GGACTTGTGGCTGGGTTT-3'	Amplification of genomic
gSQS-r2	5'-TTGTAACGGTTGGCAGAGAG-3'	sequence of
5' -R1	5'-GCTGCTGAATAACAAGACCGA-3'	<i>MsSQS</i>
5' -R2	5'-ATCTGCTTCTCGCGTTCT-3'	
g-degenerate	5'-NTCGA(G/C)T(A/T)T(G/C)G(A/T)GAA-3'	
3'-f1	5'-GCTTATCTCTGCCAACCGT-3'	

Note: restriction site of *Xho I*, *Sal I*, *Xba I* and *Bam H I* was underlined.

**Table S3.** Protein property of SQS enzymes in the 12 indicated species.

<b>Species</b>	<b>gene ID</b>	<b>cDNA (bp)</b>	<b>a.a</b>	<b>kDa</b>	<b>pI</b>
<i>M. Sativa</i>	MsSQS	1439	413	47.25	7.53
<i>M. truncatula</i>	Mt4g071520	2316	413	47.08	7.71
<i>A. thaliana</i>	At4g34640	1902	410	47.14	6.59
	At3g34650	1656	413	47.18	8.01
<i>G. max</i>	Gm12g038200	1851	413	47.11	6.57
	Gm11g112000	1898	413	47.01	6.58
<i>N. tabacum</i>	A4A49_02417	1995	411	46.98	7.86
	A4A49_25438	1353	411	47.06	8.40
	A4A49_10777	1281	403	46.04	7.25
	A4A49_12750	1285	405	47.34	7.25
<i>P. trichocarpa</i>	Pt009g123100v3	2309	413	47.31	7.29
	Pt004g161200v3	1793	476	54.69	8.88
<i>O. sativa</i>	Os03g0805100	1310	403	46.10	7.86
	Os07g0200700	1533	410	46.78	6.97
<i>Z. Mays</i>	Zm00001d013048	1398	401	46.04	6.77
	Zm00001d034516	1417	403	46.18	7.06
<i>T. aestivum</i>	Ta5A02g454500	1706	404	46.35	7.04
	Ta5B02g464600	2123	404	46.49	7.04
	Ta5D02g465000	2327	404	46.38	6.76
<i>C. reinhardtii</i>	Cr03g175250v5	2445	461	52.04	6.77
<i>H. sapiens</i>	AAP36671.1	2467	417	48.10	6.52
<i>S. Cerevisiae</i>	ACD03847.1	1350	444	51.73	5.74
<b>Average</b>		1774	416	47.68	7.20

Note: information on gene accession number is listed in the legend of Figure 1b.

**Table S4.** Prediction of the secondary structure of SQS in the indicated species.

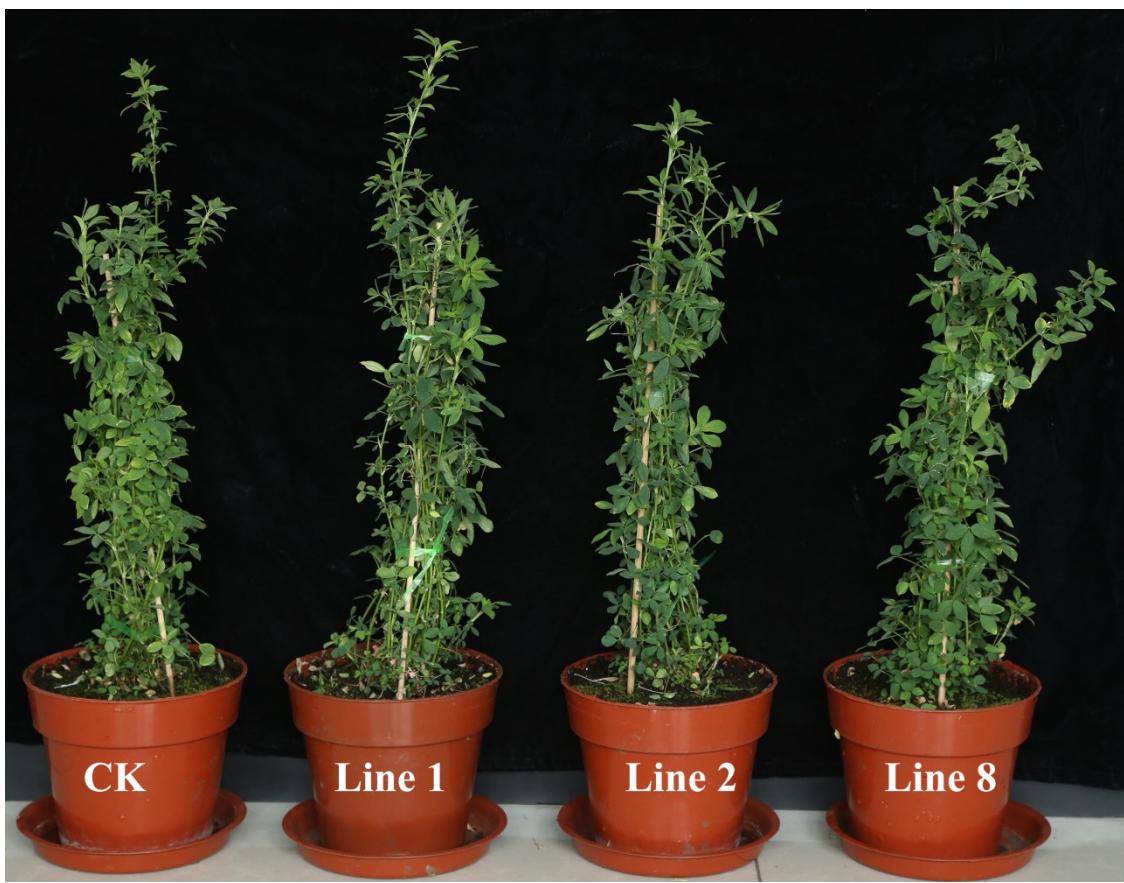
Gene ID	alpha helix	random coil	beta turn	extended strand
<b>MsSQS</b>	69.25%	22.52%	4.12%	4.12%
<b>Mt4g071520</b>	66.34%	22.52%	4.36%	6.78%
<b>At4g34640</b>	70.70%	18.03%	4.23%	7.04%
<b>At3g34650</b>	68.97%	20.00%	4.48%	6.55%
<b>Gm12g038200</b>	67.31%	24.46%	3.15%	5.08%
<b>Gm11g112000</b>	69.49%	22.28%	3.87%	4.36%
<b>A4A49_02417</b>	69.10%	20.68%	3.65%	6.57%
<b>A4A49_25438</b>	68.61%	21.65%	3.65%	6.08%
<b>A4A49_10777</b>	69.73%	23.33%	3.47%	3.47%
<b>A4A49_12750</b>	66.17%	21.98%	3.46%	8.40%
<b>Os03g0805100</b>	70.22%	19.11%	5.21%	5.46%
<b>Os07g0200700</b>	70.00%	20.49%	4.39%	5.12%
<b>Zm00001d013048</b>	67.78%	24.92%	2.74%	4.56%
<b>Zm00001d034516</b>	68.73%	23.82%	3.97%	3.47%
<b>Pt009g123100v3</b>	68.52%	22.03%	3.63%	5.81%
<b>Ta5A02g454500</b>	71.04%	20.05%	3.71%	5.20%
<b>Ta5B02g464600</b>	67.82%	21.78%	4.21%	6.19%
<b>Ta5D02g465000</b>	67.82%	22.03%	4.46%	5.69%
<b>AAP36671.1</b>	67.87%	24.22%	3.12%	4.80%
<b>ACD03847.1</b>	67.79%	24.10%	2.70%	5.41%
<b>Average</b>	68.66%	22.00%	3.83%	5.51%

information on gene accession number is listed in the legend of Figure 1b.

GCTTATTCTGTAGAAACAAAAG  
 1 ATGGGAAGTATAAAAGCGATTTGAAGAATCCAGATGATTTTCCATTACTTAAGCTGAAAATCGCGCGAGAAC  
 1 M G S I K A I L K N P D D F F P L L K L K I A A R N  
  
 79 GCCGAGAACAGATCCCACCGAGCCGCATTGGGGATTCTGTTACTCTATGCTTCATAAGGTTCTAGAACGCTCGGT  
 27 A E K Q I P P E P H W G F C Y S M L H K V S R S F G  
  
 157 CTTGTTATTCTAGCAGCTGGTCCCGAGCTCGTGTGCTGATATTCTATTGGTTCTCGTGTCTTGACACC  
 53 L V I Q Q L G P E L R D A V C I F Y L V L R A L D T  
  
 235 GTTGAGGATGATAACAAGCATAGAACAGATGTCAAGGTTCCCATACTAATGGATTTCATCGTCACATCTATGATAAT  
 79 V E D D T S I E T D V K V P I L M D F H R H I Y D N  
  
 313 GATTGGCACTTGGGTGTGGCACGAAAGAGTACAAAGTTCTAATGGACCAGTTCCATCATGTTCAAAGGCTTCTG  
 105 D W H F G C G T K E Y K V L M D Q F H H V S K A F L  
  
 391 GAACTTGGAAAGAACATATCAGGATGCAATCGAGGACATTACGAAAAGAATGGGTGCTGGAATGGCAAATTCAATTG  
 131 E L G K N Y Q D A I E D I T K R M G A G M A K F I C  
  
 469 AAGGAGGTAGAAACAATTGATGACTACGATGAATATTGCACTATGTCGGCTGGACTTGTGGCTGGTTATCAAG  
 157 K E V E T I D D Y D E Y C H Y V A G L V G L G L S K  
  
 547 CTTTTCTACGCATCTGGTAAAGAAGATCTGGCTACAGACAAACTTCAAACCTCAATGGTTGTTCTCAGAAAACC  
 183 L F Y A S G K E D L A T D K L S N S M G L F L Q K T  
  
 625 AACATTATTGAGATTATCTGAAAGACATCAATGAGATAACAAAGTCACGCATTTGGCCACGGCAGATCTGGAGT  
 209 N I I R D Y L E D I N E I P K S R M F W P R Q I W S  
  
 703 AAATATGTTATCAAACCTGGGACTTGAAATATGAGGAAAATCCGTTAAGGCTGTGCAATGCTTAAATGACATGATC  
 235 K Y V I K L E D L K Y E E N S V K A V Q C L N D M I  
  
 781 ACTAATGCTTGCTGCATGCTGAAGATTGCTTACAATACATGTCGATTACGAGACCCCTCTAATTTCGCTTTGT  
 261 T N A L L H A E D C L Q Y M S A L R D P S N F R F C  
  
 859 GCTATTCCCTCAGGTAATGCAATTGGAACACTTGCATGTGCTATAACAACATGGTGTTCAGAGGAGTAGTTAA  
 287 A I P Q V M A I G T L A M C Y N N I G V F R G V V K  
  
 937 ATGAGGCGAGGTCTAACTGCCAAAGTGATTGACCGAACAAAGACTATGGCTGATGTCTATGGTGTCTTTGATTT  
 313 M R R G L T A K V I D R T K T M A D V Y G A F F D F  
  
 1015 GCTTCCATGTTGGAGTCCAAGGTTGACAAAATGATCAAATGCAACAAAACATCGAGCAGGCTGGAAGCTATACAG  
 339 A S M L E S K V D K N D P N A T K T S S R L E A I Q  
  
 1093 AAAACTTGCAGAGAATCTGGTCTCTAACCAAAAGAAATCTTACGTTTGAGGAATGAGAGCGCATATGGCTCTACC  
 365 K T C R E S G L L T K R K S Y V L R N E S A Y G S T  
  
 1171 ATGATTCTCTTACTGGTCATCTGGTCCATCTTTTGCTTATCTCTGCTTACCGTAAACCGTACAATAACTAA  
 391 M I L L V I L L S I F F A Y L S A N R Y N N \*  
 TGTAAGTTCTATTAAATTGGTTGGTAAACAGTGTGTTGAATGTGCTAGAATGTGAGAAATTAAACAAAATACT  
 AAGTTATCCTTCTGATGAAATAGTTCAATTCTTTGTAGCAACGGTATTATGGCTTCACAAACATGGATAGCAA  
 CAGAACAGTGGTAGCAT

**Figure S1.** cDNA sequence of SQS cloned from alfalfa and the deduced protein sequence. The ORF was numbered and \* represents the stop code (TAA).





**Figure S3.** Image of 3-month-old control and the three transgenic alfalfa lines overexpressing *MsSQS*.