Supplementary Table S1. Primers used for *G. uralensis* gene cloning

Primer No.	Sequence (5' to 3')	Purpose
1	CACCATGGTGAAGATTTGCTGCATTGGA	Isolation: GuUGD1 – F
2	AGCCACTGCAGGCATGTCCTTGAGCCAA	Isolation: GuUGD1 – R
3	CACCATGGTGAAGATTTGCTGCATTGGT	Isolation: GuUGD2 – F
4	TGCCACAGCTGGCATGTCTTTGAGCCAT	Isolation: GuUGD2 – R
5	CACCATGGTGAAGATTTGCTGCATTGGTG	Isolation: GuUGD3 – F
6	GGCCACAGCTGGCATGTCTTTGAG	Isolation: GuUGD3 – R
7	CACCATGATGAAGATTTGTTGCATTGGAGC	Isolation: GuUGD4 – F
8	GGCCACTACTGCAGGCATGTCCTTGA	Isolation: GuUGD4 – R
9	CACCATGGTACTAAAGATCTGTGGAATTG	Isolation: GuUGD5 – F
10	TGCCTGCTGAGGCATGTTCTTCAGC	Isolation: GuUGD5 – R
11	AAGGTAGGCATATGGAGCTCATGGTGAAGATTTGCTGCAT	Infusion: GuUGD1 & GuUGD3 – F
12	TAGACTGCAGGTCGACAGCCACTGCAGGCATGTC	Infusion: GuUGD1 – R
13	GCACCGAGCTCATGGTGAAGATTTGCTGCAT	Infusion: GuUGD2 – F
14	ACAAAGTCGACTGCCACAGCTGGCATGTCTT	Infusion: GuUGD2 – R
15	TAGACTGCAGGTCGACGGCCACAGCTGGCATGTC	Infusion: GuUGD3 – R
16	AAGGTAGGCATATGGAGCTCATGATGAAGATTTGTTGCATTG	Infusion: GuUGD4 – F
17	TAGACTGCAGGTCGACGGCCACTACTGCAGGCAT	Infusion: GuUGD4 – R
18	AAGGTAGGCATATGGAGCTCATGGTACTAAAGATCTGTGG	Infusion: GuUGD5 – F
19	TAGACTGCAGGTCGACTGCCTGCTGAGGCATGTT	Infusion: GuUGD5 – R
20	AAGGTAGGCATATGGAGCTCATGGTGAAGATATGTTGTATTG	Infusion: AtUGD2 – F
21	TAGACTGCAGGTCGACGGCAACGGCAGGCATGTC	Infusion: AtUGD2 – R

The underlined sequences were added to facilitate unidirectional cloning of the product into pENTR/D-TOPO (Thermo Fisher Scientific).

Supplementary Table S2. GuUGDs isoforms isolated in this study

Unigene No. 9220, 9877, 6695, 13362, 14817, 7645, 16486, and 16498 were obtained from the transcriptome database. The *G. uralensis* gene ID and scaffold are described in the genome database. Unigenes 6695, 13362, 14817, 7645, 16486, and 16498 were identified as partial CDSs.

Isoform	Protein size (aa)	Unigene No.	*G. uralensis* gene ID	Scaffold
GuUGD1	481	Unigene9220	Glyur005913s00045896	Scaffold05913
GuUGD2	480	Unigene9877	Glyur000709s00024175	Scaffold00709
GuUGD3	480	Unigene6695 Unigene13362	Glyur004265s00037612	Scaffold04265
GuUGD4	481	Unigene14817 Unigene7645	Glyur000381s00016138	Scaffold00381
GuUGD5	482	Unigene16486 Unigene16498	Glyur000050s00005721	Scaffold00050

Supplementary Table S3. Primers used for *M. truncatula* gene cloning

Primer No.	Sequence (5' to 3')	Purpose
22	CACCATGGTGAAAATTTGTTGCATTGG	Isolation: MtUGD1 – F
23	TGCCACTGCAGGCATGTC	Isolation: MtUGD1 – R
24	CACCATGGTGAAGATTTGTTGCATTGG	Isolation: MtUGD2 – F
25	AGCCACTGCAGGCATGTCCTTGAGCCAA	Isolation: MtUGD2 – R
26	CACCATGGTTAAGAAGATCTGTGGAATTGG	Isolation: MtUGD3 – F
27	GCAATCCACCCACTGGTCTATTGG	Isolation: MtUGD3 – R
28	AAGGTAGGCATATGGAGCTCATGGTGAAAATTTGTTGCATTG	Infusion: MtUGD1 – F
29	TAGACTGCAGGTCGACTGCCACTGCAGGCATGTC	Infusion: MtUGD1 – R
30	AAGGTAGGCATATGGAGCTCATGGTGAAGATTTGTTGCATTG	Infusion: MtUGD2 – F
31	TAGACTGCAGGTCGACAGCCACTGCAGGCATGTC	Infusion: MtUGD2 – R
32	AAGGTAGGCATATGGAGCTCATGGTTAAGAAGATCTGTGGAA	Infusion: MtUGD3 – F
33	TAGACTGCAGGTCGACGCAATCCACCCACTGGTC	Infusion: MtUGD3 – R

The underlined sequences were added to facilitate unidirectional cloning of the product into pENTR/D-TOPO (Thermo Fisher Scientific).

Supplementary Table S4. Primers used for qRT-PCR

Primer No.	Sequence (5’ to 3’)	Purpose
34	GATTGAGGTAGCCGTGGTCG	qRT-PCR: GuUGD1 – L
35	CTTCCCCTGCACTCCTTCAC	qRT-PCR: GuUGD1 – R
36	ACTGTCCCTGTCAAAACCGC	qRT-PCR: GuUGD2 – L
37	TTGATTGCAGTTCCCTCAGCA	qRT-PCR: GuUGD2 – R
38	CTGGGGATACAAGGGAGACA	qRT-PCR: GuUGD3 – L
39	AAGCATCCCAAACCACACTC	qRT-PCR: GuUGD3 – R
40	AGGGGCTGTTGGGAGATAGG	qRT-PCR: GuUGD4 – L
41	GGGGCTTGGTGGTTGAAGAT	qRT-PCR: GuUGD4 – R
42	TCACTCCCGCATATCAGCTTG	qRT-PCR: GuUGD5 – L
43	CCTCACACACATGCTTCTCCA	qRT-PCR: GuUGD5 – R
44	TCTTCGCAAACTGGCAGTGA	qRT-PCR: GuTubulin – L
45	CGAGATGTGAGTGGGGCAAA	qRT-PCR: GuTubulin – R
46	GGTGGTTTATCAGCGTGGGA	qRT-PCR: GubAS – L
47	TGCTCAACTACAATGTCCGCA	qRT-PCR: GubAS – R
48	ATGGACGAAAATTGGAGGACGA	qRT-PCR: GuCYP88D6 – L
49	CTGGTTGCTGTACTTTCATGGC	qRT-PCR: GuCYP88D6 – R
50	ATGGCGACCCTTACAAGCTC	qRT-PCR: GuCYP72A154 – L
51	AGATTCGTGGTGCAGCATCA	qRT-PCR: GuCYP72A154 – R
52	CCAAGCACTCTACAAACTCTCCA	qRT-PCR: GuCYP93E3 – L
53	GGATTTGCTTAGCCATTTCTGCA	qRT-PCR: GuCYP93E3 – R
54	CAGGGGAGCCTAGTAACAATGAC	qRT-PCR: GuCYP72A566 – L
55	GCCCTGCCAAGTAAAATAGCTTC	qRT-PCR: GuCYP72A566 – R
56	GTGGTGCTTACTTGGCTGGA	qRT-PCR: GuUGT73P12 – L
57	GCCAACGACCCAAATGAAATCA	qRT-PCR: GuUGT73P12 – R

Supplementary Table S5. Correspondence between the accession number in GenBank and the gene model name in SoyBase or probe set ID in MtGEA

G. max and *M. truncatula UGD* nucleotide sequences that correspond to nucleotide sequences in GenBank were retrieved from SoyBase_Wm82.a1.v1.1 (https://www.soybase.org/) and MtGEA_ver.3 (https://mtgea.noble.org/v3/), respectively, by blastn similarity search. No homologous nucleotide sequence to XP_003607793 was found in the database. Protein size were calculated based on isolated MtUGD isoforms.

Glycine max	
GenBank accession number	SoyBase gene model name (SoyBase_Wm82.a1.v1.1)
NP_001304614	Glyma01g06970
XP_003518763	Glyma02g12870
NP_001238410	Glyma08g26520
XP_003552559	Glyma18g50000
XP_003543940	Glyma13g06050
XP_003554799	Glyma19g03500
XP_003525453	Glyma05g00590
XP_003549560	Glyma17g08490

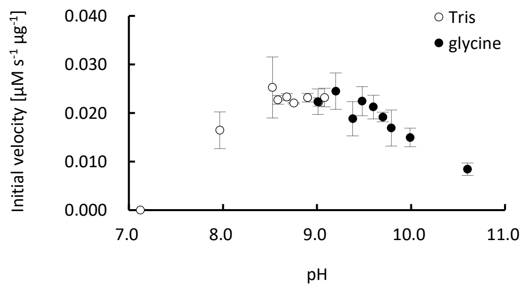
Medicago truncatula			
Isoform	Protein size (aa)	GenBank accession number	MtGEA probe set ID (MtGEA ver. 3)
MtUGD1	480	XP_003607793	-
MtUGD2	480	XP_003621403	Mtr.48440.1.S1_at
MtUGD3	478	XP_013457966	Mtr.41235.1.S1_at
-	-	XP_003614035	Mtr.25689.1.S1_at

Supplementary Table S6. Identities between the X-ray crystal homo-hexamer structure of human UGD and GuUGDs

Isoform	Template			Sequence				Quality	
	SMTL ID	Biounit Oligo State	Method	Seq identity [%]	Seq similarity	Range	Coverage	QSQE	GMQE
GuUGD2	6c4j.2	Homo-hexamer	X-ray, 2.53 Å	61.46	0.48	1 – 469	0.97	0.63	0.78
GuUGD5	6c4j.2	Homo-hexamer	X-ray, 2.53 Å	59.44	0.47	2 – 471	0.97	0.62	0.78

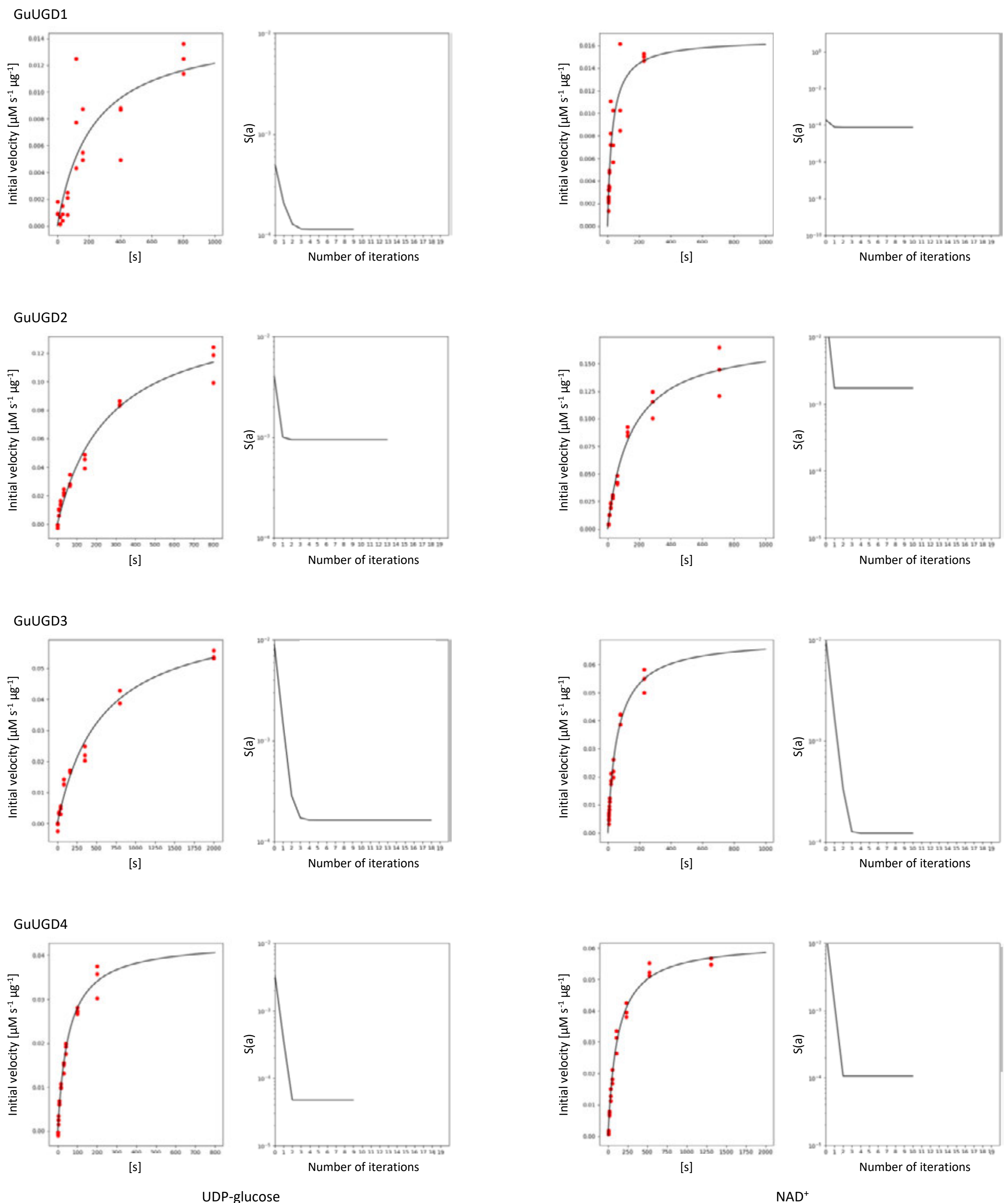
Supplementary Table S7. Amino acid sequence identity among MtUGD1–3 and GuUGDs

	MtUGD2	MtUGD3	GuUGD1	GuUGD2	GuUGD3	GuUGD4	GuUGD5
MtUGD1	85 %	74 %	90 %	86 %	87 %	99 %	81 %
MtUGD2		74 %	87 %	95 %	92 %	85 %	82 %
MtUGD3			74 %	75 %	75 %	74 %	82 %



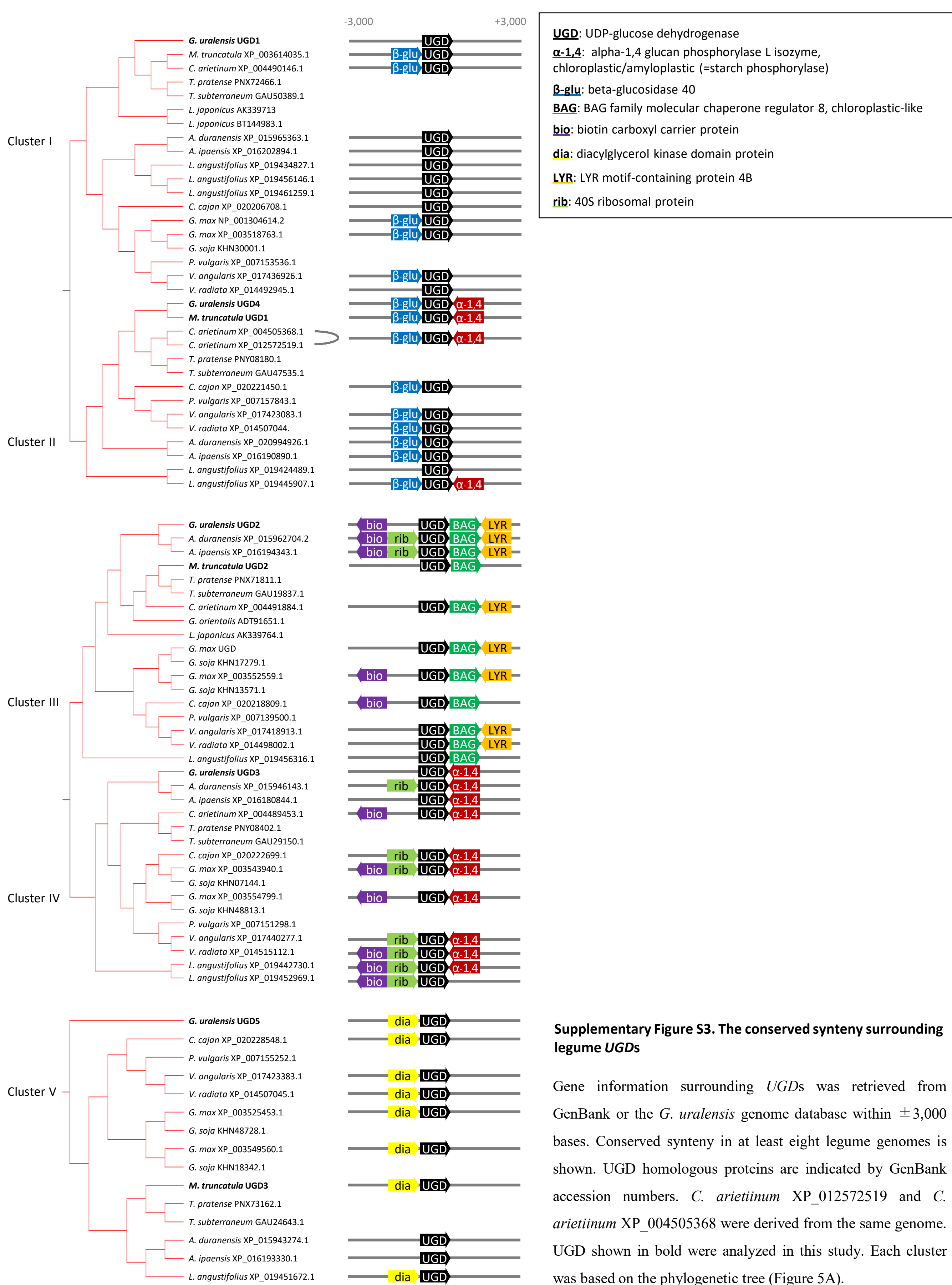
Supplementary Figure S1. Enzyme activity under various pH conditions

0.5 μg GuUGD4, 500 μM UDP-glucose, and 1,000 μM NAD^+ were used in this assay. Values represent the mean ± SD of three measurements.



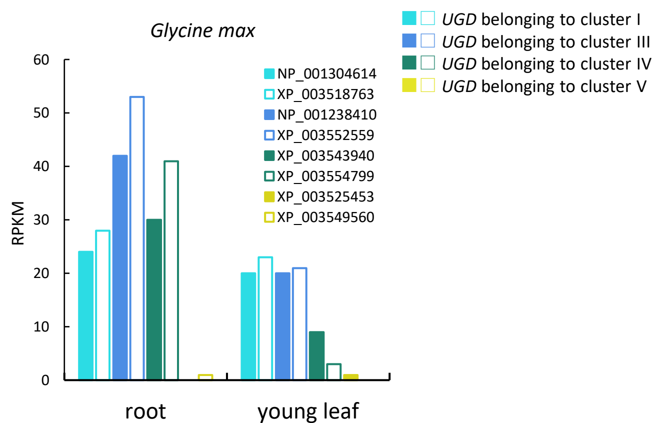
Supplementary Figure S2. Kinetic analyses of GuUGDs

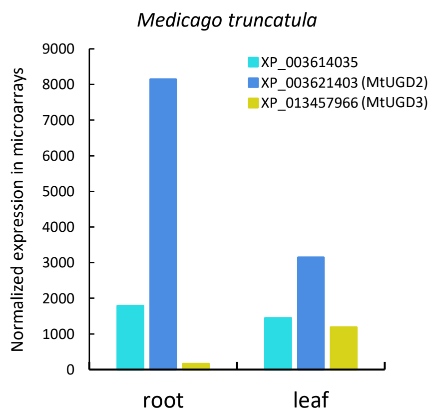
The kinetic parameters of the recombinant GuUGDs were determined for both the substrate UDP-glucose and the cofactor NAD^+. Left panels indicate the results of curve fitting based on the Michaelis-Menten equation by the Gauss-Newton method. Right panels indicate the relationship between the number of iterations and the sum of squares of fitting parameters. Convergence to any sum of squares value suggests a successful fit.



Supplementary Figure S3. The conserved synteny surrounding legume *UGDs*

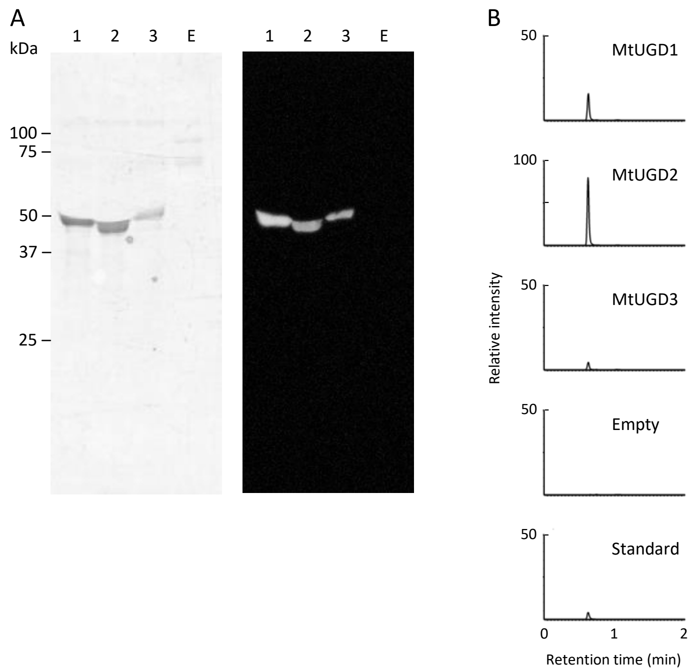
Gene information surrounding *UGDs* was retrieved from GenBank or the *G. uralensis* genome database within ±3,000 bases. Conserved synteny in at least eight legume genomes is shown. UGD homologous proteins are indicated by GenBank accession numbers. *C. arietiinum* XP_012572519 and *C. arietiinum* XP_004505368 were derived from the same genome. UGD shown in bold were analyzed in this study. Each cluster was based on the phylogenetic tree (Figure 5A).





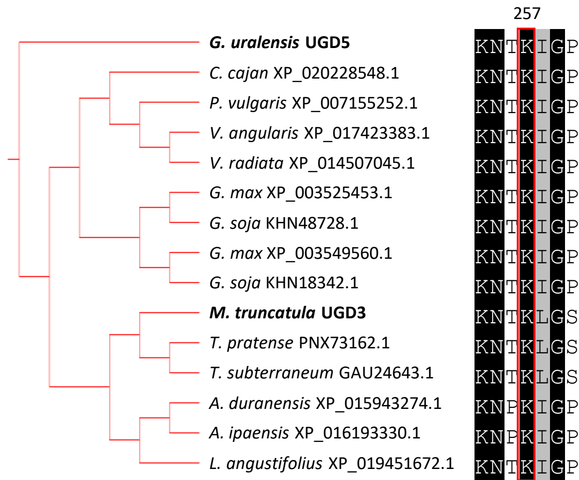
Supplementary Figure S4. Expression of legume *UGD*s

Expression of *UGD*s is based on RPKM values (*G. max*) or normalized expression in microarrays (*M. truncatula*). Transcript levels of *M. truncatula UGD*s are based on the fluorescence intensity of each hybridized DNA probe, which was performed using global normalization (Benedito et al. 2008). *UGD* genes belonging to clusters I, III, IV, and V are light blue, blue, green, and yellow, respectively. Number correspondence between GenBank, each database and isolated isoforms is displayed in Supplementary Table S5.



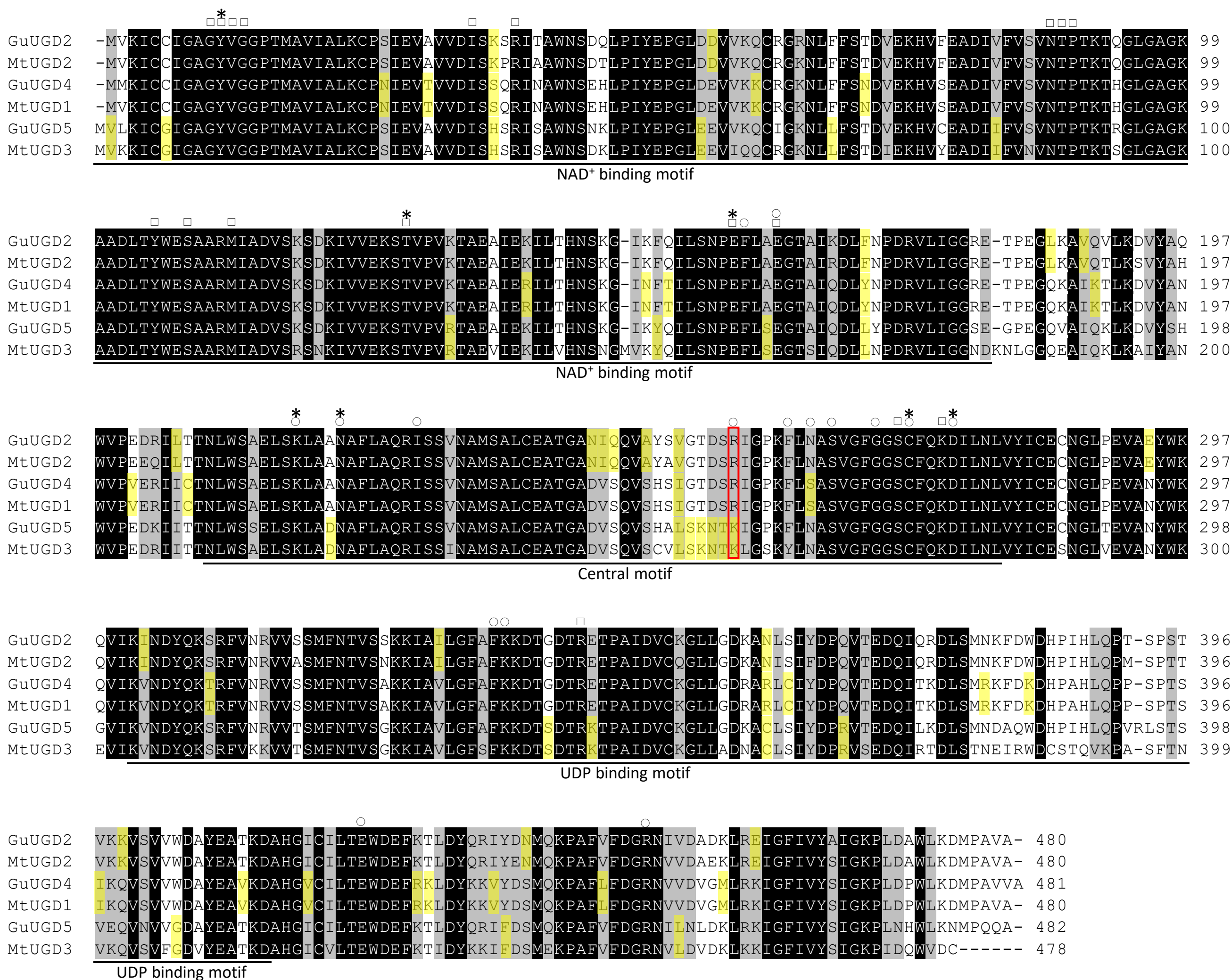
Supplementary Figure S5. UGD activity of purified MtUGD recombinant proteins

(A) Electrophoresis of purified recombinant proteins. Purified His-tagged recombinant proteins were detected in a UV-stained SDS-PAGE gel (left panel) and by anti-His detection in a Western blotting membrane transferred from an identical gel (right panel). UGDs were detected as ~50 kDa. **Lanes 1–3:** purified recombinant MtUGD1–3 proteins (6 µl of MtUGD1–3 solutions were loaded, respectively); **E:** protein solution removed non-interactive protein for the TALON resin, extracted from IPTG-induced *E. coli* transformed with empty vector (6 µl of protein solution was loaded). (B) UPLC-MS chromatograms at *m/z* 579.1 for the *in vitro* reaction products. All reaction products were diluted 10 times after 1 day of incubation using 1 µg of each purified UGD (protein solution extracted from *E. coli* transformed with empty vector was used at the same volume used for UGD-containing protein solutions). 100 % corresponds to the intensity indicated in products catalyzed by MtUGD2. UDP-glucuronic acid was used as an authentic standard.



Supplementary Figure S6. A unique amino acid residue of Fabales UGDs belonging to cluster V

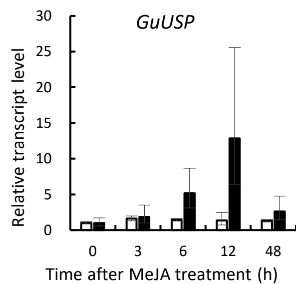
Parts of the aligned amino acid sequences of Fabales UGDs belonging to cluster V (based on the phylogenetic tree; Figure 5A) are shown. The alignment was performed using BioEdit with ClustalW. Amino acid residues framed in red likely decreased attraction to UDP-glucose.



* proposed as important residues for catalysis in previous reports
○ shown as having UDP-glucose interaction in SWISS-MODEL
□ shown as having NAD⁺ interaction in SWISS-MODEL

Supplementary Figure S7. Amino acid sequences of GuUGD2, GuUGD4, GuUGD5 and MtUGD1–3

Alignment of amino acid sequences deduced from cDNA of cloned GuUGD2, GuUGD4, GuUGD5 and MtUGD1–3. MtUGD belonging to the same cluster as the GuUGD was displayed below the GuUGD sequence. We made the alignment using BioEdit with ClustalW. The NAD^+ binding motif, UDP binding motif, and central motif were annotated based on GenBank. Asterisks indicate key residues for catalysis proposed in previous studies (Campbell et al. 2000; Egger et al. 2011); circles and squares indicate amino acids shown as key residues interacting with UDP-glucose and NAD^+, respectively, by SWISS-MODEL. Amino acid residues shown in yellow background were specific to the cluster. The amino acid residue framed in red was the same position as shown in Figure 2.



Supplementary Figure S8. Quantitative expression analyses (qPCRs) of *USP* transcripts

Transcript levels in mock-treatment tissue-cultured stolons are indicated by outlined bars; MeJA treatments are indicated by filled bars. Relative transcript levels 0 h after treatment were set equal to 1. Error bars indicate the SD of three technical replicates.