Appendix A

Soybean transformation method

Agrobacterium preparation

Agrobacterium single clones were picked from the streak plate and placed into a 10 ml tube containing YEP medium plus antibiotics. The cultures were shaken at 28℃ and 250 rpm overnight to reach saturation (density above OD_{600}=1.2). One millilitre of the total culture was added to 100 ml fresh YEP medium containing antibiotics in a 250 ml flask. The cultures were shaken overnight at 28℃ and 250 rpm until the mixture reached a concentration of OD_{600}=1.0–1.2. The cultures were centrifuged at 3500 rpm at RT for 10 min, and the pellets were resuspended thoroughly with co-cultivation medium containing 1/10 Gamborg's B5 salts (Gamborg *et al.* 1968), 1/10 MS iron, 3% sucrose, 20 mM MES (pH5.4), 1/10 B5 vitamins, 7.4 µM 6-Benzylaminopurine (BAP), 0.7 µM GA3, and 400 µM acetosyringone to obtain a final OD_{600} = 0.6 by gentle shaking.

Transformation of soybean

Approximately 100 soybean seeds were placed in each petri plate, placed into desiccators inside a fume hood and sterilized using chlorine (Zhang *et al.* 1999). The seeds were germinated in germination medium (GM) containing B5 salts, B5 vitamins, MS iron, 2% sucrose, and 0.8% Agar (pH 5.8) for 4–5 days depending on the genotype. The germinating seeds were excised using a $^{\#}$15 scalpel blade approximately 3–4 mm from the hypocotyls; then vertically cut through the hypocotyl and split the two cotyledons apart evenly. The cotyledons were soaked in the prepared *Agrobacterium* in a petri plate, the tips of the epicotyls were removed, and the cotyledonary node region was wounded by gently making several slices. Sliced cotyledons were immediately placed onto another plate within the *Agrobacterium* suspension and inoculated for 30 min with occasional agitation. Solid co-cultivation medium was covered with a piece of sterile filter paper, 6 explants were placed onto the surface with the wounded side down. The plates were sealed with venting tape and placed in a Percival chamber (Percival) for 4–5 d with an 18/6 h light/dark cycle at 24°C.

After a brief rinse in washing medium (WM) containing B5 salts, MS iron, 2% sucrose, 3 mM MES (pH 5.7), B5 vitamin, 100 mg/L timentin, 100 mg/L cefotaxime, and 100 mg/L carbenicillin, the explants were sub-cultured on shoot induction medium (SIM) containing B5 salts, MS iron, 2% sucrose, 3 mM MES (pH 5.7), 1 μM BAP, 5μM Kinetin, B5 vitamin, 50 mg/L timentin, 100 mg/L cefotaxime, 100 mg/L carbenicillin，and 2 mg/L glufosinateammonium (Olhoft *et al.* 2003)，for two weeks, at which time the glufosinateammonium concentration was increased from 2 mg/L to 8 mg/L for another two weeks. After shoot induction, the explants were transferred to shoot elongation medium containing MS salts, MS iron, 3% sucrose, 3 mM MES (pH 5.7), B5 vitamin, 50 mg/L Asp, 50 mg/L Glu, Asp 50 mg/L, 0.5 mg/L GA3, 1 mg/L Zeatin riboside, timentin, 50 mg/L cefotaxime, 100 mg/L carbenicillin，4 mg/L glufosinateammonium, and agar.

The plantlets were excised when they reached approximately 3–4 cm, and were dipped into 1 m/ml IBA for 1–3 min. Then, the plantlet was transferred to rooting medium containing MS salts, MS with iron, 2% sucrose, 3 mM MES (pH 5.7), B5 vitamins, 50 mg/L Asp, 50 mg/L Glu and 0.8% agar for rooting. After the root was generated, the plantlets were transferred to a compound matrix (vermiculite-soil, 1:1) for growth.

Supporting References

1. Gamborg O L, Miller R A, and Ojima K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research.* **50,** 151-158.
2. Olhoft P M, Flagel L E, Donovan C M and Somers D A. 2003. Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method. *Planta*, **216**:723-735.
3. Zhang Z, Xing A, Staswick P, and Clemente T E. 1999. The use of glufosinate as a selective agent in *Agrobacterium*-mediated transformation of soybean. *Plant Cell Tissue and Organ Culture*, **56**, 37-46.

Appendix B

Table Soluble amino acid contents in mature dry seeds of wild-type ZD soybean cultivar and transgenic soybean lines expressing the *AtD-CGS* gene. The quantities of amino acids were analyzed using GC-MS, and their levels were normalized to norleucine and calculated as nmol per g fresh weight of seeds. Statistically significant changes (*P* <0.05, using two-way ANOVA) were identified by asterisk.

Table. Seed soluble amino acids content in ZD transgenic soybean and ZD wild type

Amino Acid	**ZD-CK**	**ZD-36-1**	**ZD-36-2**	**ZD-42-1**	**ZD-42-2**
Alanine	312.6±99.6	355.3±60.7	315.4±11.5	320.2±98.0	311.9±92.7
Valine	129.6±77.7	169.5±27.2	149.2±6.7	126.6±73.7	218.5±11.5
Serine	99.3±17.3	73.2±9.2	46.1±5.0	101.5±68.7	86.7±9.2
Leucine	81.0±50.4	119.0±18.9	121.7±12.8	98.9±67.9	160.1±37.4
Threonine	156.0±27.3	154.2±31.4	115.2±3.0	153.4±2.5	155.5±3.1
Isoleucine	142.4±73.1	179.9±17.3	195.5±12.0	162.7±76.7	248.0±10.7
Methionine	113.4±39.9	141.27±11.7	105.2±7.1	108.5±2.5	123.0±24.3
Proline	65.6±81.3	147.2±23.0	123.0±4.6	121.1±18.6	143.8±5.5
Glycine	393.8	458.1±338.0	265.4±56.3	419.8±118.4	506.8±168.4
Homoserine	3.3±1.3	4.1	3.1	4.4±1.0	7.1
Aspartate	1188.9±173.2	1651.1±230.9*	1475.5±49.2*	1359.5±145.3*	1248.7±260.4*
Phenylalanine	454.3±12.4	472.0±39.5*	527.7±27.9*	464.0±30.5*	436.8±33.6
Glutamate	2243.9±152.5	2961.1±465.0	2526.3±52.5	2487.0±317.0	2290.6±305.8
Lysine	27.8±7.7	22.6±1.7*	21.9±1.1*	32.9±1.5*	25.0±1.5*
Tyrosine	224.4±30.7	198.9±22.1	205.6±9.8	210.5±48.5	237.1±37.4
Tryptophan	1186.8±484.9	1508.0±92.2	1202.9±106.1	1389.0±376.7	1039.0±20.6

Appendix C

Table Total amino acid contents in mature dry seeds of wild-type ZD soybean cultivar and transgenic soybean lines expressing the *AtD-CGS* gene. The quantities of amino acids were analyzed using GC-MS, and their levels were normalized to norleucine and calculated as nmol per g fresh weight of seeds. Statistically significant changes (*P* <0.05, using two-way ANOVA) were identified by asterisk.

Table. Seed hydrolyzed amino acids content in ZD transgenic soybean and ZD wild type

Amino Acid	ZD-WT	ZD-36-1	ZD-36-2	ZD-42-1	ZD-42-2
Alanine	33377±20141	39911±15925*	53619±38500 *	31875±20495	78243±1230 *
Valine	14544±12224	16758±8121	25139±19653	12054±9667	74835±6514 *
Serine	38633±23645	45784±22559	51738±36457 *	39844±36559	100314±4789 *
Leucine	31175±22576	34807±14090	51077±39257	25679±17521	124911±4260 *
Threonine	18773±13894	23420±12688	37073±29240	16810±15877	92078±3772 *
Isoleucine	15702±12961	17725±8315	26175± 20163	12326±9821	72320±3727*
Methionine	4288±1923	6446±2586*	12561±1927*	8531 ± 153*	20557±3772*
Proline	42365±26670	49906±21299	66839±49843 *	37572±25746	106899±1980 *
Glycine	91602±27465	83075±20545	90031±45290	96809±55375	121618±8236
Aspartate	93326±36859	104529±31956 *	108119±56633*	61305±16918	168236±8426 *
Phenylalanine	27276±13397	32570±10304	40023±28097	24190±18300	73429±5073 *
Glutamate	145147±121108	168456±120763	182694±144841	91958±86522*	354564±13727*
Histidine	8165±4724	16571±7127 *	28585± 4353 *	14838*	23738±2447 *
Lysine	42541±27177	52316±20065	66793±50512	41281±32296	128238±3527 *
Tyrosine	12488±8759	15737±6793	21437±16364 *	12358±10977	39645±741 *

Appendix D

Table Soluble amino acid contents in mature dry seeds of wild-type JX51 soybean cultivar and transgenic soybean lines expressing the *AtD-CGS* gene. The quantities of amino acids were analyzed using GC-MS, and their levels were normalized to norleucine and calculated as nmol per g fresh weight of seeds. Statistically significant changes (*P* <0.05, using two-way ANOVA) were identified by asterisk.

Table. Seed soluble amino acids content in JX51 transgenic soybean and JX wild type

Amino acid	JX-WT	JX-102	JX-107	JX-112	JX-113	JX-119
Homoserine	14.6±7.4	81.9±25.3 *	48.7±22.7 *	75.9±21.8 *	118.3±54.8 *	171.6±108.7 *
Asparagine	2588.1±364.3	9166.5±751.4 *	4677.3±164.9 *	7606.9±4804.1	5671.8±1648.9 *	9184±1546.7 *
Glutamine	11.1±12.8	34.3±1.7 *	26.2±9	27.2±9.5	21.4±1.8	34.5±13.8 *
Lysine	100.1±40.7	236.9±27.4 *	161.9±39.6 *	185.2±79	175.7±21 *	229.7±67.9 *
Serine	199.2±48.1	367.3±28.5 *	312.6±110.1 *	429.6±147.7 *	455.9±161.4 *	423.2±76.5 *
Aspartate	440.3±281.7	732.1±82.6	609.6±93.5	653.3±180	538.3±31.6	703.7±87.2
Threonine	110.1±23.6	172.6±12.1 *	131.4±34.8	170.6±61.4	148.2±23.7 *	157.3±20.1 *
Cysteine	41.4±24.6	64.4±8.6	42.9±10.9	67.5±31.7	44.1±1.7	59.2±6.3
Tyrosine	406.1±222.5	622.3±469.7	596.7±260.5	266.8±55.4	221.6±59.9	190.6±50.1
Isoleucine	2092±397.1	2882.8±172.7 *	2424.6±279.4	2956.8±533*	2611.1±128.7 *	2773.9±247 *
Methionine	37.3±18.9	136.8±11.8 *	99.3±22.9 *	140.6±60.5 *	123.6±39.5 *	163.9±13.5 *
Glutamate	263.3±114	357.8±27.8	225.5±37.6	287.5±143.6	357.5±81.4	390.1±79.8
Valine	78.2±20.9	100.5±11.3	79.8±14.1	93.4±28.8	84.6±6.6	94.3±16.7
Alanine	222.9±64.5	282.7±44.6	189.3±68.2	287.1±127.9	548.6±267.4 *	383.6±70.2 *
Phenylalanine	63.5±35.3	75.4±8.5	65.9±10.7	70.9±18.3	63±9.6	75.6±14.5
Leucine	69.7±18.4	74.4±6.9	60.9±14.9	73±23.9	68.4±5	76.1±12.8
Proline	97.2±27.1	99.5±4.9	77.1±8.5	103.4±29.3	87.7±8.7	96±22.5
Glycine	201.3±93.8	196.6±69.9	165.7±60.9	173.5±66.5	239.7±68.9	244.8±87.2
Tryptophan	323±188.8	265.2±59.5	475.2±157.6	338.6±120.9	253±67	398±176.4

Appendix E

Table Total amino acid contents in mature dry seeds of wild-type JX51 soybean cultivar and transgenic soybean lines expressing the *AtD-CGS* gene. The quantities of amino acids were analyzed using GC-MS, and their levels were normalized to norleucine and calculated as nmol per g fresh weight of seeds. Statistically significant changes (*P* <0.05, using two-way ANOVA) were identified by asterisk.

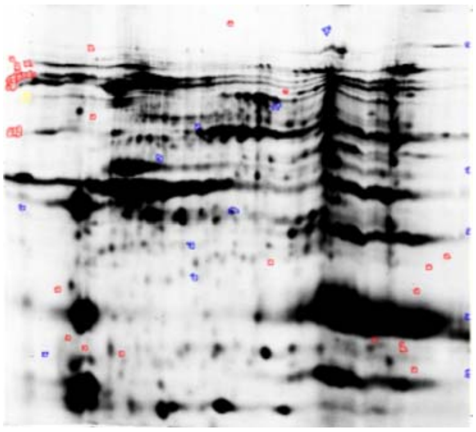
Table. Seed hydrolyzed amino acids content in JX51 transgenic soybean and JX wild type

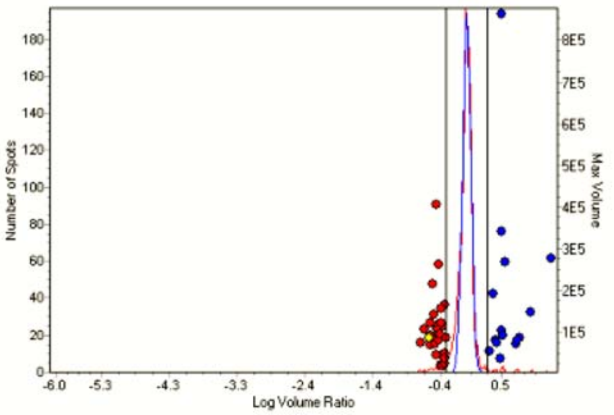
Amino acid	WT	102	107	112	113	119
Alanine	58731.4±23719	52833±24344	45726±7604	43776±15999	57885±21093	51871±131
Valine	18996±4246	22542±9944	21583±2417	18897±7414	17420±4192	20707±375
Serine	33132±5235	46602±16547*	44441±5706*	59912±25329*	44308±12113	59512±110
Leucine	16821±3552	18060±4325	17734±2120	17540±8091	15022±3759	15431±658
Threonine	16353±3695	20798±4811*	20070±1499*	23178±10489	17165±4099	21014±399
Isoleucine	13382±2952	18929±4587*	16535±1635*	18096±7783	14903±3948	17163±346
Methionine	7552.6±1937	12289±6144*	12722±1347*	13234±5892*	10006±2689*	17677±371
Proline	20788±6537	23473±15296	23290±3544	28716±15103	19513±5673	23824±596
Glycine	237473±282072	139445±140293	199564±247642	86083±24914	63984±26068	67006±452
Aspartate	36136±33995	40854±21138	49434±7030	61971±39550	28314±25723	72876±185
Phenylalanine	20419±3735	20829±5231	23844±4425	21595±4984	21364±3177*	21577±438
Glutamate	42185±15984	30240±17876	17649±14268	18180±23917	29781±17877	35332±161
Asparagine	60869±36426	463599±217405*	166791±45789*	286430±176295*	260326±110874*	408046±13
Lysine	15856±5803	23482±8966	18153±5670	13293±8391	15126±7551	17694±613
Tyrosine	7902±3081	10280±5879	8785±2707*	5919±4150	7017±3726	7357±2810

Appendix F

Protein profile detection with difference gel electrophoresis

Total seed protein from wild type (ZD) and transgenic soybean (ZD-36) was labeled with Cy3 and Cy5, respectively, while the pooling sample (WT and transgenic soybean) was labeled with Cy2. A total of 2290 spots were identified, and 1635 spots were included, of which 31 spots decreased and 15 spots increased. The distribution of the different spots showed no significant changes in the expression of major seed storage proteins. Increased spots were labeled blue and decreased spots were labeled red.





Appendix G

Germination rate determination

Seeds of transgenic and wild type lines were grown in sand bed in green house. For each line, 50 seeds were randomly selected as a repeat one of three repeats for testing. During the generation, the sand bed was irrigated to keep wet. The germination rate was calculated and analyzed using ANOVA.

Table Germination rate of transgenic ZG, JX and wild type

Line	Germination rate % (Mean ± SD)
ZD-36	100.0±0.0
ZD-42-1	98.7±1.2
ZG-WT	99.4±1.2
JX-51	96.0±2.0
JX-WT	96.7±1.2