

Table 1. Samples used in this study

Sample no.	Name of cultivar
A	Product of Canada
B	(OAC Kent [Ippanhakumokudaizu])
C	Product of USA
D	(GL3494 [Tamabijin])
E	Jack
F	
G	Williams (Wild-type)
H	
I	Williams (GM)
J	

Table 2. Evaluation of total RNA purified

Sample no.	Total volume (μL)	Concentration ($\text{ng}/\mu\text{L}$)	Total amount obtained (μg)	Absorbance ratio		RIN
				A_{260}/A_{280}	A_{260}/A_{230}	
A	47	802	37.69	2.06	1.65	8
B	40	764	30.56	2.19	2.06	7.9
C	39	794	30.97	2.20	1.37	7.7
D	45	396	17.82	2.17	1.91	7.2
E	41	176	7.22	2.24	0.37	7.1
F	44	210	9.24	2.17	1.60	8.4
G	44	52	2.29	2.21	1.45	7.1
H	42	108	4.54	2.20	0.49	7.6
I	45	404	18.18	2.17	1.18	7.5
J	45	280	12.60	2.17	0.82	7.5

Table 3. Evaluation of RNA-Seq reads

Sample no.	Raw paired reads	Clean paired reads	Effective rate (%)	Q20 (%)	Total single reads	Single reads mapped in pairs	Single reads mapped in broken pairs	Single reads not mapped
A	51,441,409	50,729,766	98.62	97.79	101,459,532	61,672,590 (60.79%)	28,691,993 (28.28%)	11,094,949 (10.94%)
B	49,683,359	49,016,783	98.66	97.68	98,033,566	56,209,740 (57.34%)	29,043,653 (29.63%)	12,780,173 (13.04%)
C	44,531,292	44,034,849	98.89	97.73	88,069,698	46,011,594 (52.24%)	30,690,052 (34.85%)	11,368,052 (12.91%)
D	47,882,082	47,317,833	98.82	97.69	94,635,666	52,819,398 (55.81%)	29,460,833 (31.13%)	12,355,435 (13.06%)
E	61,319,942	60,574,989	98.79	98.12	121,149,978	69,789,842 (57.61%)	38,011,810 (31.38%)	13,348,326 (11.02%)
F	48,122,897	47,577,266	98.87	98.38	95,154,532	55,738,078 (58.58%)	28,605,777 (30.06%)	10,810,677 (11.36%)
G	45,459,810	43,843,369	96.44	98.12	87,686,738	51,498,634 (58.73%)	25,660,013 (29.26%)	10,528,091 (12.01%)
H	55,188,240	53,372,808	96.71	98.14	106,745,616	61,030,950 (57.17%)	33,180,743 (31.08%)	12,533,923 (11.74%)
I	58,862,750	56,974,554	96.79	98.19	113,949,108	65,403,516 (57.40%)	35,492,083 (31.15%)	13,053,509 (11.46%)
J	49,725,464	49,153,651	98.85	97.64	98,307,302	58,565,980 (59.57%)	29,295,701 (29.80%)	10,445,621 (10.63%)

Table 4. List of differential genes between Non-GM-type and Williams cultivar*

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA06G44091.1	6	47071378..47075313	1.76E-46	613.48
GLYMA12G34075.2	12	37241711..37243166	7.72E-34	444.24
GLYMA17G31900.1	17	35078089..35079829	1.98E-31	412.01
GLYMA17G20450.1	17	19471430..19474526	3.16E-30	395.89
GLYMA20G01430.1	20	962695..963815	1.26E-29	387.83
GLYMA12G00991.1	12	542484..543426	6.47E-27	355.59
GLYMA17G33075.1	17	36682979..36684646	2.65E-23	307.24
GLYMA10G32820.1	10	41214830..41215683	4.24E-22	291.12
GLYMA16G22474.1	16	25971208..25973609	1.70E-21	283.06
GLYMA03G19460.1	3	24727108..24729564	1.70E-21	283.06
GLYMA09G08330.1	9	7472274..7473626	2.71E-20	266.95
GLYMA04G05040.1	4	3784240..3786870	2.71E-20	266.95
GLYMA10G22390.2	10	28500279..28506009	2.71E-20	266.95
GLYMA05G33481.1	5	38116443..38119643	1.09E-19	258.89
GLYMA09G35840.1	9	41727496..41729798	4.34E-19	250.83
GLYMA16G34840.1	16	37366383..37367407	1.74E-18	242.77
GLYMA16G33750.1	16	36551930..36553977	2.78E-17	226.65
GLYMA11G22373.1	11	19578803..19579699	2.78E-17	226.65
GLYMA17G35105.1	17	39104206..39105398	2.78E-17	226.65
GLYMA20G05250.1	20	5992299..5994285	2.78E-17	226.65

*Twentieth most different expression level genes are listed

Table 5. List of differential genes between non-GM- and GM-type Williams cultivar*

Feature ID	Chromo	Region	P-value	Fold change
GLYMA11G14040.2	11	10078670..10082298	9.72E-12	519.19
GLYMA17G02260.1	17	1453405..1456485	7.18E-14	418.87
GLYMA07G32100.2	7	36998904..36999990	6.50E-09	333.37
GLYMA06G01391.1	6	866877..869760	1.62E-06	276.46
GLYMA09G03090.1	9	2178668..2180818	2.79E-07	231.93
GLYMA11G12770.1	11	9120544..9124159	7.15E-07	201.67
GLYMA17G08051.1	17	5950558..5951790	1.98E-07	194.5
GLYMA01G44640.2	1	55233128..55234930	8.31E-07	188.1
GLYMA13G42100.1	13	42191818..42193149	3.87E-05	185.59
GLYMA17G03480.2	17	2316582..2318978	2.90E-07	184.55
GLYMA09G28575.1	9	35505909..35507116	1.16E-04	177.78
GLYMA13G09707.1	13	11308589..11311684	8.47E-07	172.61
GLYMA14G17863.1	14	19931432..19937024	1.63E-04	171.31
GLYMA12G15951.1	12	14972263..14973737	4.60E-07	164.74
GLYMA01G40460.1	1	52219822..52222031	7.03E-06	156.28
GLYMA05G10075.1	5	10080145..10085029	1.75E-05	148.59
GLYMA02G13800.2	2	12123744..12127608	8.76E-06	140.3
GLYMA02G13800.1	2	12123744..12127608	5.71E-05	134.32
GLYMA12G33711.1	12	36913314..36916524	9.98E-06	134.16
GLYMA08G06330.1	8	4487558..4489934	8.60E-06	133.2

*Twentieth most different expression level genes are listed

Table 6. Gene lists having >600 times differential gene expression level between US and Canada cultivar using US cultivar as a reference

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA20G01945.1	20	1467724..1469637	9.58E-23	15,220.93
GLYMA09G16090.1	9	19201065..19203101	2.47E-25	14,006.49
GLYMA06G10840.1	6	8215351..8217052	4.27E-08	8,690.69
GLYMA14G33161.2	14	40620309..40624931	3.34E-07	6,197.73
GLYMA03G03451.1	3	3197245..3198319	1.38E-07	5,411.74
GLYMA14G23895.1	14	28387656..28392096	2.11E-06	3,855.04
GLYMA08G10460.1	8	7589047..7589812	6.03E-07	2,422.29
GLYMA09G04500.2	9	3316706..3319908	2.65E-15	1,730.21
GLYMA19G31674.1	19	39473386..39476650	1.83E-06	1,516.51
GLYMA06G02170.1	6	1433698..1435146	5.77E-13	1,358.87
GLYMA08G22640.2	8	17188397..17189549	7.18E-14	1,339.68
GLYMA10G38440.1	10	46239325..46240490	3.31E-08	1,311.02
GLYMA09G05660.1	9	4446091..4450837	2.06E-10	1,176.59
GLYMA17G26556.1	17	27707046..27712214	2.34E-05	1,104.14
GLYMA14G33161.1	14	40620309..40624930	9.32E-07	1,094.60
GLYMA17G19007.1	17	16773936..16774706	2.30E-12	1,026.82
GLYMA01G06250.2	1	6303784..6304910	6.98E-09	958.13
GLYMA03G08170.2	3	9017226..9018873	2.69E-05	934.17
GLYMA12G14980.1	12	13759744..13760377	2.00E-11	922.42
GLYMA20G27410.1	20	36585232..36588760	2.18E-22	834.97
GLYMA10G31991.1	10	40469872..40471094	6.28E-11	790.3
GLYMA19G34210.1	19	41826839..41830086	3.08E-23	774.34
GLYMA19G26435.1	19	33159771..33163336	2.13E-14	757.64
GLYMA07G33511.1	7	38428874..38432132	7.89E-15	721.56
GLYMA15G05391.1	15	3808741..3815631	4.47E-08	720.97
GLYMA17G13820.1	17	10616633..10617551	1.47E-10	716.37
GLYMA10G13450.1	10	15138921..15139984	2.32E-10	696.05
GLYMA14G15605.1	14	16729271..16738914	4.37E-08	684.1
GLYMA15G05391.2	15	3808944..3815631	5.09E-08	682.81
GLYMA18G19100.2	18	20858053..20865070	3.46E-05	653.36
GLYMA01G11640.1	1	14972819..14973806	8.89E-10	628.88
GLYMA01G42500.2	1	53760884..53761948	1.08E-08	613.82

Table 7. Gene lists having >600 times differential gene expression level between US and Williams cultivar using US cultivar as a reference

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA09G16090.1	9	19201065..19203101	3.72E-27	6,920.15
GLYMA20G01945.1	20	1467724..1469637	5.78E-20	1,884.69
GLYMA03G03451.1	3	3197245..3198319	7.01E-14	1,818.09
GLYMA18G19100.2	18	20858053..20865070	7.99E-19	1,577.05
GLYMA05G36750.2	5	40529554..40531831	2.13E-17	1,447.52
GLYMA04G10621.1	4	8833901..8835822	1.02E-06	1,446.89
GLYMA17G26556.1	17	27707046..27712214	1.11E-17	1,376.39
GLYMA14G12170.1	14	10900868..10903684	3.29E-13	1,288.85
GLYMA14G33161.2	14	40620309..40624931	2.84E-16	1,179.78
GLYMA05G25340.1	5	31481126..31482429	7.31E-13	945.94
GLYMA19G31674.1	19	39473386..39476650	5.52E-14	829.77
GLYMA15G39520.1	15	46174815..46178992	8.98E-14	804.45
GLYMA15G39510.1	15	46171119..46171865	3.86E-13	735.45
GLYMA17G19007.1	17	16773936..16774706	2.07E-12	695.49

Table 8. Gene lists having >600 times differential gene expression level between US and Jack cultivar using US cultivar as a reference

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA09G16090.1	9	19201065..19203101	5.24E-25	8,765.89
GLYMA20G01945.1	20	1467724..1469637	4.33E-19	4,306.51
GLYMA08G10460.1	8	7589047..7589812	2.54E-20	2,366.75
GLYMA17G26556.1	17	27707046..27712214	3.34E-19	2,088.06
GLYMA12G14980.1	12	13759744..13760377	1.01E-08	1,895.40
GLYMA05G36750.2	5	40529554..40531831	5.05E-17	1,799.88
GLYMA18G08015.1	18	6758624..6760903	6.17E-17	1,636.07
GLYMA18G03640.3	18	2502550..2503445	9.57E-10	1,427.06
GLYMA18G19100.2	18	20858053..20865070	6.08E-13	1,395.99
GLYMA08G22640.2	8	17188397..17189549	1.63E-08	1,390.54
GLYMA16G24590.1	16	28569677..28572015	1.34E-16	1,360.38
GLYMA19G31674.1	19	39473386..39476650	3.75E-15	1,218.72
GLYMA05G25340.1	5	31481126..31482429	2.22E-15	1,148.69
GLYMA07G25536.1	7	27944328..27951602	2.45E-13	1,026.02
GLYMA07G21018.1	7	21452281..21452961	1.63E-14	1,011.88
GLYMA17G19007.1	17	16773936..16774706	7.77E-13	1,010.06
GLYMA10G38440.1	10	46239325..46240490	6.13E-08	1,006.76
GLYMA07G17000.2	7	16666326..16668062	5.77E-14	929.67
GLYMA09G04500.2	9	3316706..3319908	7.25E-08	925.72
GLYMA02G03250.1	2	2518997..2520592	4.23E-19	882.39
GLYMA19G26435.1	19	33159771..33163336	1.27E-21	844.81
GLYMA18G50595.1	18	59676735..59681611	4.08E-12	700.38
GLYMA20G27410.1	20	36585232..36588760	7.99E-25	657.15
GLYMA09G29250.1	9	36191240..36192018	5.22E-10	619.38

Table 9. Gene lists having >600 times differential gene expression level between Williams and US cultivars using Williams cultivar as a reference

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA03G07121.1	3	7458627..7491220	2.93E-30	9,818.73
GLYMA15G15140.1	15	11581588..11582282	3.16E-17	1,472.81
GLYMA12G09400.1	12	7168483..7172381	8.33E-18	1,403.12
GLYMA03G06976.1	3	7275123..7298068	2.94E-16	1,145.18
GLYMA01G30060.1	1	40769687..40771162	1.67E-15	1,108.67
GLYMA14G17863.1	14	19931432..19937024	6.86E-15	1,091.65
GLYMA17G20770.1	17	19844171..19845334	8.68E-14	1,081.14
GLYMA03G05650.1	3	5984323..5988698	7.75E-15	997.9
GLYMA08G03381.2	8	2382841..2385344	5.42E-15	989.8
GLYMA05G36950.1	5	40688684..40690756	2.11E-10	661.24
GLYMA20G03670.1	20	3517516..3519869	3.47E-11	637.15

Table 10. Gene lists having >600 times differential gene expression level between Williams and Canada cultivars using Williams cultivar as a reference

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA12G13090.1	12	11449237..11449791	1.68E-32	12,060.30
GLYMA06G10840.1	6	8215351..8217052	1.77E-08	8,091.06
GLYMA12G09400.1	12	7168483..7172381	3.50E-23	3,565.85
GLYMA03G07121.1	3	7458627..7491220	2.43E-20	3,367.50
GLYMA01G30060.1	1	40769687..40771162	5.66E-24	3,340.16
GLYMA11G34613.1	11	36389844..36393874	2.43E-17	1,394.44
GLYMA14G23895.1	14	28387656..28392096	3.75E-06	1,338.77
GLYMA13G37101.1	13	38277892..38278797	6.96E-17	1,317.92
GLYMA01G31890.1	1	43130698..43137550	4.61E-05	927.18
GLYMA20G27410.1	20	36585232..36588760	7.11E-31	925.68
GLYMA01G06250.2	1	6303784..6304910	9.75E-10	854.93
GLYMA03G08170.2	3	9017226..9018873	1.59E-05	826.91
GLYMA01G18990.1	1	23424892..23425203	1.05E-26	805.92
GLYMA03G05730.2	3	6051893..6063501	1.09E-04	732.43
GLYMA10G13450.1	10	15138921..15139984	1.41E-11	633.67

Table 11. Gene lists having >600 times differential gene expression level between Williams and Jack cultivars using Williams cultivar as a reference

Feature ID	Chromosome	Region	P-value	Fold change
GLYMA02G03250.1	2	2518997..2520592	7.72E-32	21,151.66
GLYMA0892S50.1	scaffold_892	2250..3229	6.22E-28	18,414.07
GLYMA03G07121.1	3	7458627..7491220	4.34E-34	7,673.40
GLYMA02G10320.1	2	8186068..8188789	1.74E-20	5,717.26
GLYMA12G13090.1	12	11449237..11449791	4.04E-22	3,568.04
GLYMA01G30060.1	1	40769687..40771162	4.43E-27	2,907.23
GLYMA11G15310.1	11	10952617..10954051	4.80E-24	2,846.41
GLYMA12G09400.1	12	7168483..7172381	2.49E-22	2,070.67
GLYMA02G03230.1	2	2494913..2496466	3.60E-22	1,546.78
GLYMA18G08015.1	18	6758624..6760903	4.96E-20	1,541.70
GLYMA03G05730.2	3	6051893..6063501	2.72E-08	1,372.22
GLYMA13G37101.1	13	38277892..38278797	7.53E-12	1,305.76
GLYMA16G24590.1	16	28569677..28572015	1.10E-19	1,278.84
GLYMA08G03381.2	8	2382841..2385344	2.16E-15	842.83
GLYMA20G27410.1	20	36585232..36588760	1.78E-35	746.13
GLYMA03G06976.1	3	7275123..7298068	2.18E-13	660.36

Table 12. RT PCR primer sets used to analyze gene GLYMA17G18930.1 and gene GLYMA12G09400.1

FeatureID	Primer sequence (5'→3')	Amplicon (bp)
GLYMA17G18930.1	sense TTCCCTGTTGGATTTGAGAAGCTAGGAAAAG anti- CCAAAGCACAACTTCATGTTTCAGCACCAGC	349
GLYMA12G09400.1	sense GAGAGAAGGTTTTGGATTCCAACCTCCTGCAC anti- GACTGCAAATGTTTTCCCACATTTTCTGCAC	342
<i>Actin1</i> (XM_003552652)	sense ATCTTGACTGAGCGTGGTTATTCC anti- GCTGGTCCTGGCTGTCTCC	126

Table 13. Number of identified peptides and proteins

	Sequence database			
	CDS (<i>Glycine max</i>)	Uniprot (<i>Glycine max</i>)	Uniprot/SwissProt (Green Plants)	NCBI/Genome (<i>Glycine max</i>)
Number of peaks with identified peptide	3,179	3,274	1,844	3,205
Number of identified proteins	451	457	289	450
Number of proteins that are quantitatively analyzable	424	429	244	420

Table 14. Protein fold change of more than 4 between Jack and Williams

No.	Protein name	Uniprot AccessionNumber	Gene Name	Confidence Score	Unique Peptide Counts	Normalized abundance		Fold Change Jack/Williams
						Williams	Jack	
1	Uncharacterized protein	I1NDJ4	GLYMA_20G027200	42.3	1	0	55,840	infinity
2	Beta-conglycinin, beta chain	P25974	CG-4	4975.0	4	5,093,644	1,087,833,393	213.57
3	Uncharacterized protein	I1MG41	GLYMA_15G119800	38.5	1	28,809	2,906,869	100.90
4	Uncharacterized protein	I1L3Q2;K7LE73	GLYMA_09G158600	219.9	3	4,098,609	283,449,042	69.16
5	Uncharacterized protein	I1LY75	GLYMA_13G111700	32.7	1	8,134	303,090	37.26
6	Uncharacterized protein	I1LRP2	GLYMA_12G097400	39.4	1	424,319	5,112,728	12.05
7	Uncharacterized protein	I1JCB6	GLYMA_02G042500	41.9	1	416,136	3,630,876	8.73
8	Uncharacterized protein	K7KLI9	GLYMA_04G216900	24.6	1	393,577	3,236,300	8.22
9	Uncharacterized protein	I1MT39	GLYMA_17G075800	25.3	1	36,737	175,677	4.78
10	Uncharacterized protein	I1N3G2;I1L6T4	GLYMA_18G222200	57.7	2	615,022	2,930,016	4.76
11	Uncharacterized protein	C6SWF6;I1L3J0	GLYMA_16G202500	99.3	2	350,671	1,593,327	4.54
12	Uncharacterized protein	C6SYG6	GLYMA_19G236100	74.4	1	1,283,949	5,683,054	4.43
13	Uncharacterized protein	I1L5X5	GLYMA_09G238000	39.6	1	75,873	328,339	4.33
14	Alpha-1,4 glucan phosphorylase	I1KYU6	GLYMA_08G334000	28.5	1	31,889	135,800	4.26
15	Uncharacterized protein	K7KVC7	GLYMA_06G160300	42.0	1	178,246	757,851	4.25
16	Alpha-1,4 glucan phosphorylase	I1LWR4	GLYMA_13G057800	48.4	2	63,829	270,360	4.24

Table 15. Protein fold change of less than 0.25 between Jack and Williams

No.	Protein name	Uniprot AccessionNumber	Gene Name	Confidence Score	Unique Peptide Counts	Normalized abundance		Fold Change Jack/Williams
						Williams	Jack	
1	Uncharacterized protein	K7N4I7		314.4	1	10,655	0	0.00
2	Uncharacterized protein	I1N2I4	GLYMA_18G182600	45.2	1	53,895	0	0.00
3	Glycinin G3	P11828	GY3	3452.8	25	2,839,742,376	331,645,220	0.12
4	G.max mRNA from stress-induced gene (H4)	Q43453	GLYMA_17G030200	116.2	1	686,801	145,678	0.21
5	Uncharacterized protein	I1JPV1	GLYMA_03G189300	24.1	1	146,880	31,709	0.22

Table16. Protein fold change of more than 4 between GM and WT Williams

No.	Protein name	Uniprot AccessionNumber	Gene Name	Confidence Score	Unique Peptide Counts	Normalized abundance		Fold Change GM/Williams
						Williams	GM	
1	Uncharacterized protein	I1NDJ4	GLYMA_20G027200	1	42.32	0	59,882	Infinity
2	SYNC1 Soybean	I1K000;I1KPU2;I1K0Q4	Sync1	5	237.86	49,680	14,909,520	300.11
3	Uncharacterized protein	I1LY75	GLYMA_13G111700	1	32.68	8,134	481,321	59.17
4	Enhanced green fluorescent protein(eGFP)	X1XX11	eGFP	8	583.25	8,473,101	462,686,460	54.61
5	Uncharacterized protein	K7N4I7		1	314.43	10,655	189,604	17.79
6	Uncharacterized protein	C6SWF6;I1L3J0	GLYMA_16G202500	2	99.26	350,671	3,558,002	10.15
7	Uncharacterized protein	I1MG41	GLYMA_15G119800	1	38.51	28,809	287,122	9.97
8	Uncharacterized protein	I1MT39	GLYMA_17G075800	1	25.27	36,737	275,245	7.49
9	Uncharacterized protein	K7KLI9	GLYMA_04G216900	1	24.58	393,577	2,753,076	7.00
10	Uncharacterized protein	I1N3G2;I1L6T4	GLYMA_18G222200	2	57.69	615,022	4,233,763	6.88
11	Uncharacterized protein	I1L3Q2;K7LE73	GLYMA_09G158600	3	219.91	4,098,609	26,178,334	6.39
12	Uncharacterized protein	A0A0R0GZ14;A0A0R4J3J4;A0A0R0I189	GLYMA_13G176500	1	89.69	116,136	630,523	5.43
13	Uncharacterized protein	I1L0N6		1	52.61	291,650	1,497,587	5.13
14	Uncharacterized protein	I1JBN4;C6TFW8	GLYMA_02G019600	2	86.54	578,708	2,910,093	5.03
15	Uncharacterized protein	C6SYG6	GLYMA_19G236100	1	74.35	1,283,949	6,432,535	5.01
16	Alpha-mannosidase	I1ML97;I1JXQ5		2	77.18	420,057	1,930,058	4.59
17	Superoxide dismutase	I1JYA9	GLYMA_04G221300	1	40.22	14,174	64,430	4.55
18	Uncharacterized protein	I1LRP2	GLYMA_12G097400	1	39.41	424,319	1,909,809	4.50
19	G.max mRNA from stress-induced gene (H4)	Q43453	GLYMA_17G030200	1	116.21	686,801	3,008,123	4.38
20	Uncharacterized protein	A0A0R0GA14;A0A0R0J5K1	GLYMA_14G063800	1	100.61	588,700	2,516,664	4.27
21	Uncharacterized protein	I1NH02	GLYMA_20G164700	3	106.52	4,954,399	20,068,875	4.05

Table 17. Protein fold change of less than 0.25 between GM and WT Williams

No.	Protein name	Uniprot AccessionNumber	Gene Name	Confidence Score	Unique Peptide Counts	Normalized abundance		Fold Change Jack/Williams
						Williams	GM	
1	DHAR class glutathione S-transferase	C6TGU0	DHAR3	1	80.86	161,915	0	0.00
2	Uncharacterized protein	C6SVF6	GLYMA_16G072400	1	34.23	656,925	0	0.00
3	Acyl-[acyl-carrier-protein] desaturase	B8XJY3	SAD1	1	79.4	208,272	3,366	0.02
4	Uncharacterized protein	I1JXJ3	GLYMA_04G195100	1	250.28	1,307,307	46,826	0.04
5	Uncharacterized protein	I1KAK6	GLYMA_06G123700	1	36.1	703,867	28,449	0.04
6	Glycinin G3	P11828	GY3	25	3452.76	2,839,742,376	195,798,455	0.07
7	Uncharacterized protein	I1KVT5	GLYMA_08G224100	1	46.39	202,364	14,872	0.07
8	Uncharacterized protein	I1MGM0	GLYMA_15G148900	1	58.63	1,338,846	122,214	0.09
9	Polyadenylate-binding protein	I1JE12	GLYMA_02G103900	1	68.43	568,208	53,096	0.09
10	Uncharacterized protein	K7LKW2		1	53.15	1,522,954	184,174	0.12
11	Uncharacterized protein	I1JE09;C6T5U0	GLYMA_02G103600	2	71.88	798,138	139,381	0.17
12	60S ribosomal protein L27	I1KRI7	GLYMA_08G087200	1	25.18	1,070,867	193,508	0.18
13	Uncharacterized protein	A0A0R4J5A5	GLYMA_14G176900	1	82.46	304,399	55,076	0.18
14	Annexin	G3E7M9	GLYMA_13G088700	1	84.06	1,853,384	343,251	0.19
15	Tau class glutathione S-transferase	I1MJ34	GSTU48	1	73.69	373,220	70,822	0.19
16	Uncharacterized protein	I1MAC4	GLYMA_14G117700	1	172.26	2,912,510	648,510	0.22
17	Uncharacterized protein	A0A0R0FE22	GLYMA_17G166400	1	19.69	165,078	40,880	0.25
18	Ferritin	C6TCN5;C6TI81;I1JL80; I1MYZ8;K7MTN2;I1JL8 1;K7KA92	GLYMA_18G205800	2	107.2	7,262,792	1,822,452	0.25
19	Uncharacterized protein	I1JVB9;A0A0R0J4X0;Q 96450	GLYMA_04G099900	1	113.69	1,179,122	299,188	0.25

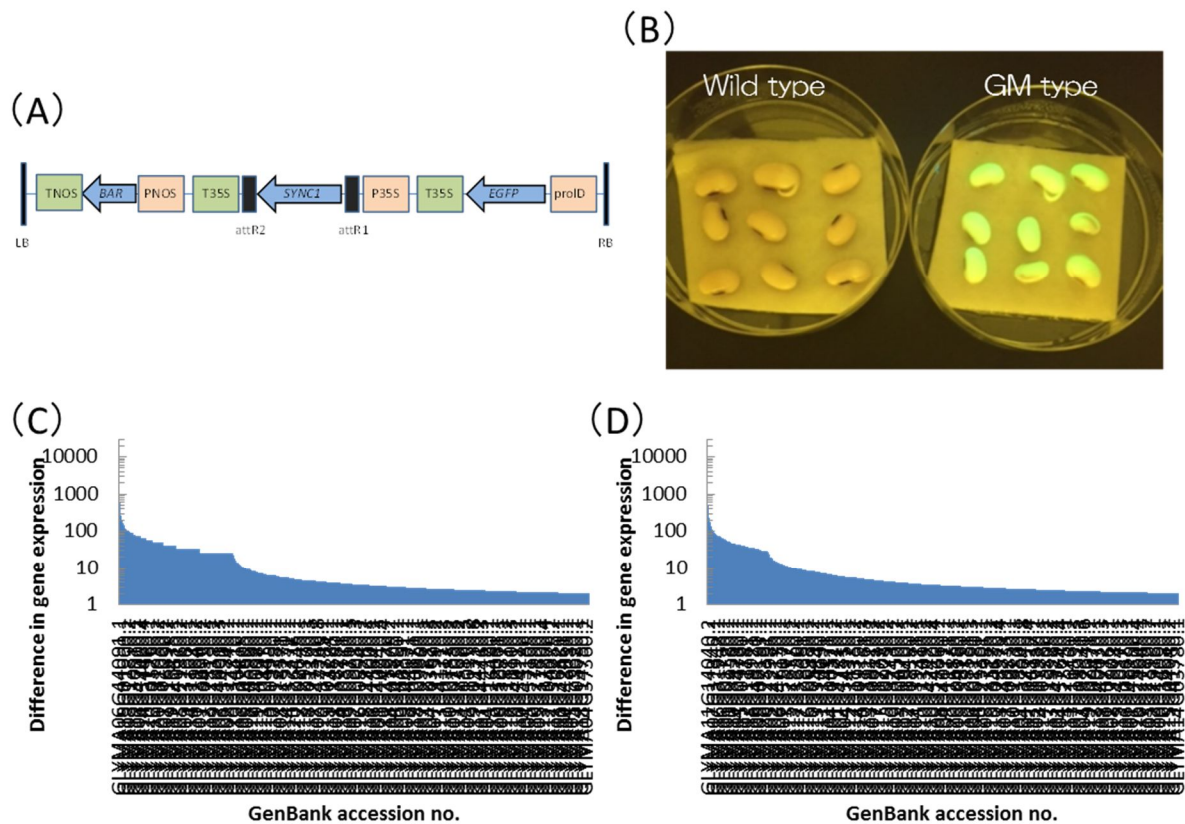


Figure 1. Identification of genes having differential gene expression between Non-GM and GM type soybean seedlings

(A) Transgenic construct in GM type (LB, left-border sequences; TNOS, nos terminator; BAR, bialaphos resistance; PNOS, nos promoter; T35S, 35S RNA terminator; attR2, recombination site; SYNC1, G. max SYNC1; attR1, recombination site, P35S, 35S RNA promoter; EGFP, ever green fluorescent protein; proID, roID promoter; RB, right-border sequences)

(B) Non-GM and GM type soybean seedlings under the UV light

Differential gene expression level between (C) Non-GM vs Non-GM type, (D) Non-GM vs GM type

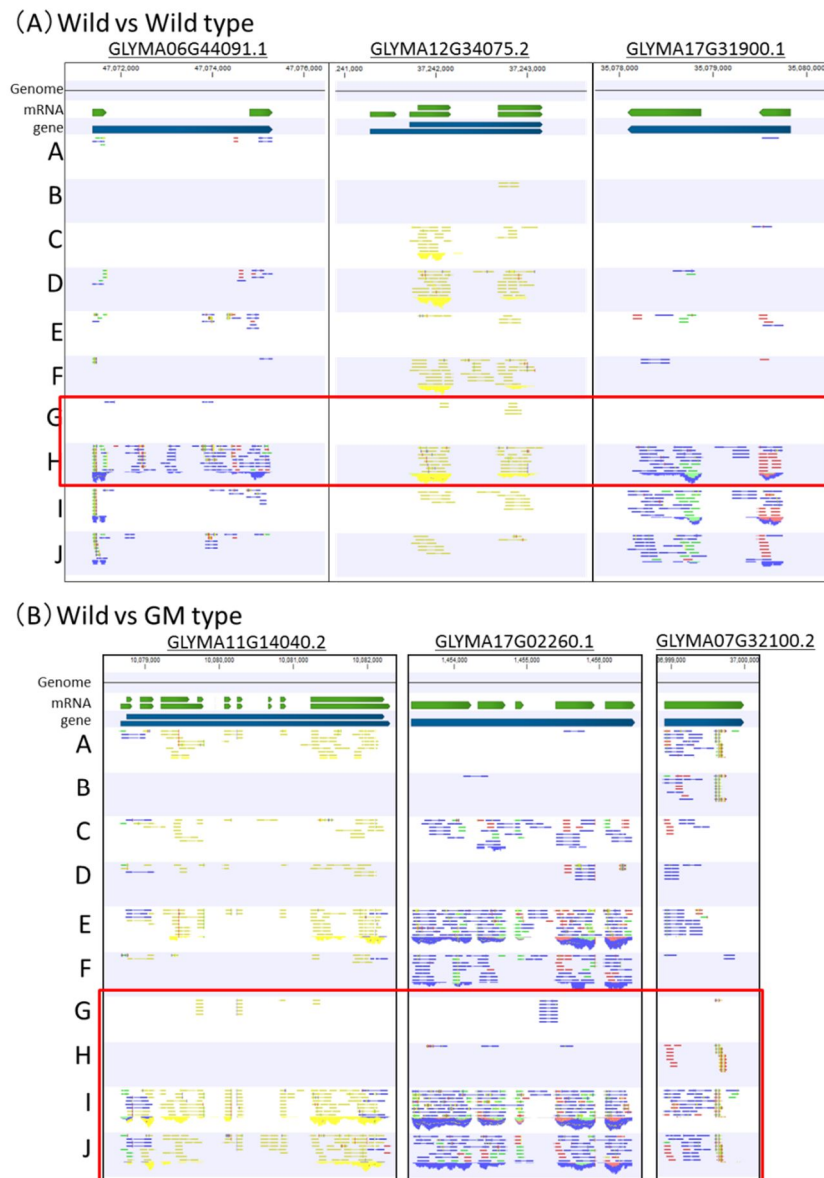


Figure 2. Mapped reads to the reference genome
Top three genes identified having gene expression difference among (A) non-GM-type Williams cultivars and (B) non-GM- and GM-type Williams cultivars

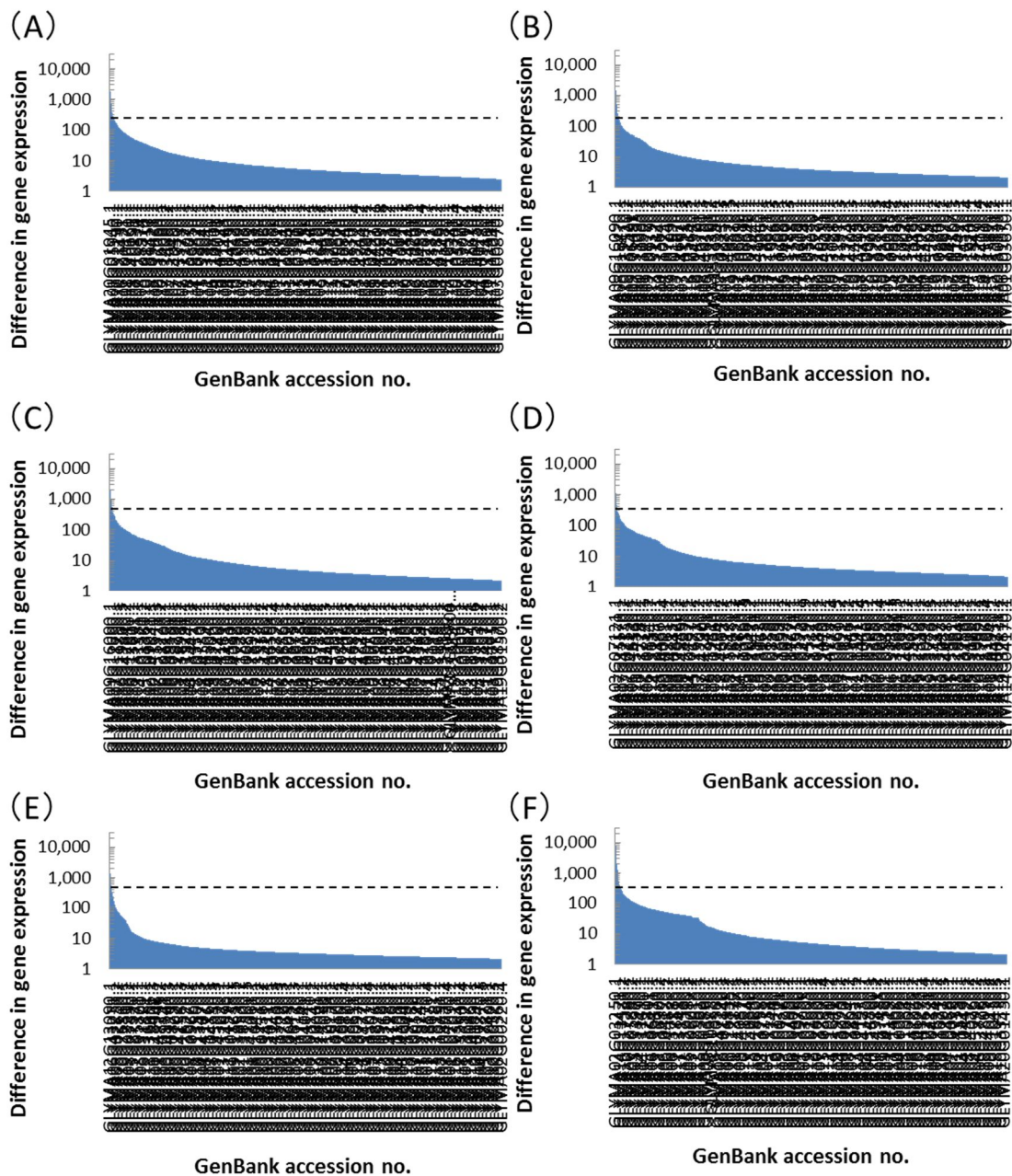


Figure 3. Differential genes expression among different varieties

Using US variety as a reference (A-C), (A) US vs Canada cultivar, (B) US vs Williams cultivar, (C) US vs Jack cultivar

Using Williams cultivar as a reference (D-F), (D) Williams vs US cultivar, (E) Williams vs Canada cultivar, (F) Williams vs Jack cultivar

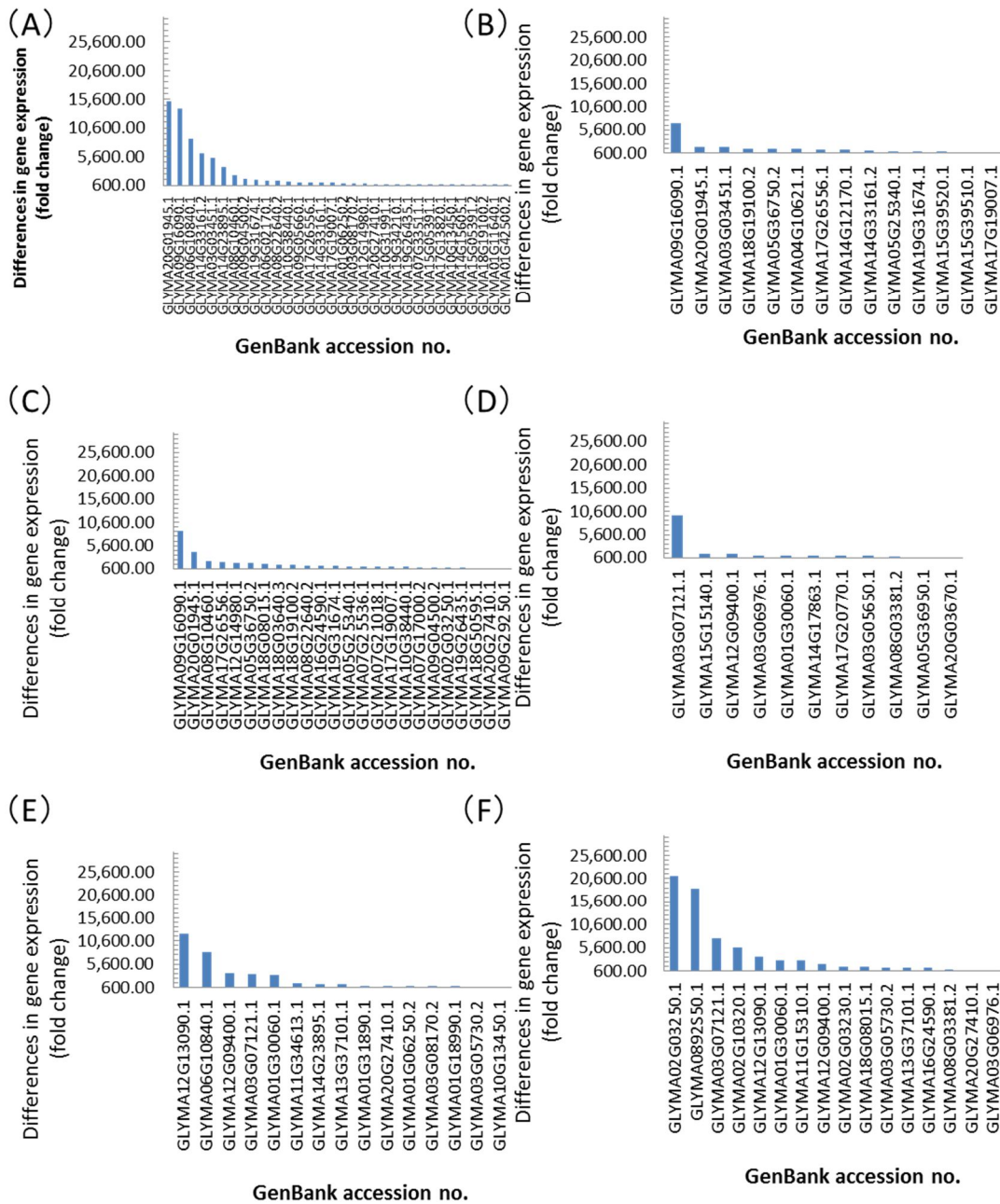


Figure 4. Identification of genes having differential gene expression >600 times using Empirical Analysis of DGE

Using US cultivar as a reference (A-C), (A) US vs Canada cultivar, (B) US vs Williams cultivar, (C) US vs Jack cultivar

Using Williams cultivar as a reference (D-F), (D) Williams vs US cultivar, (E) Williams vs Canada cultivar, (F) Williams vs Jack cultivar

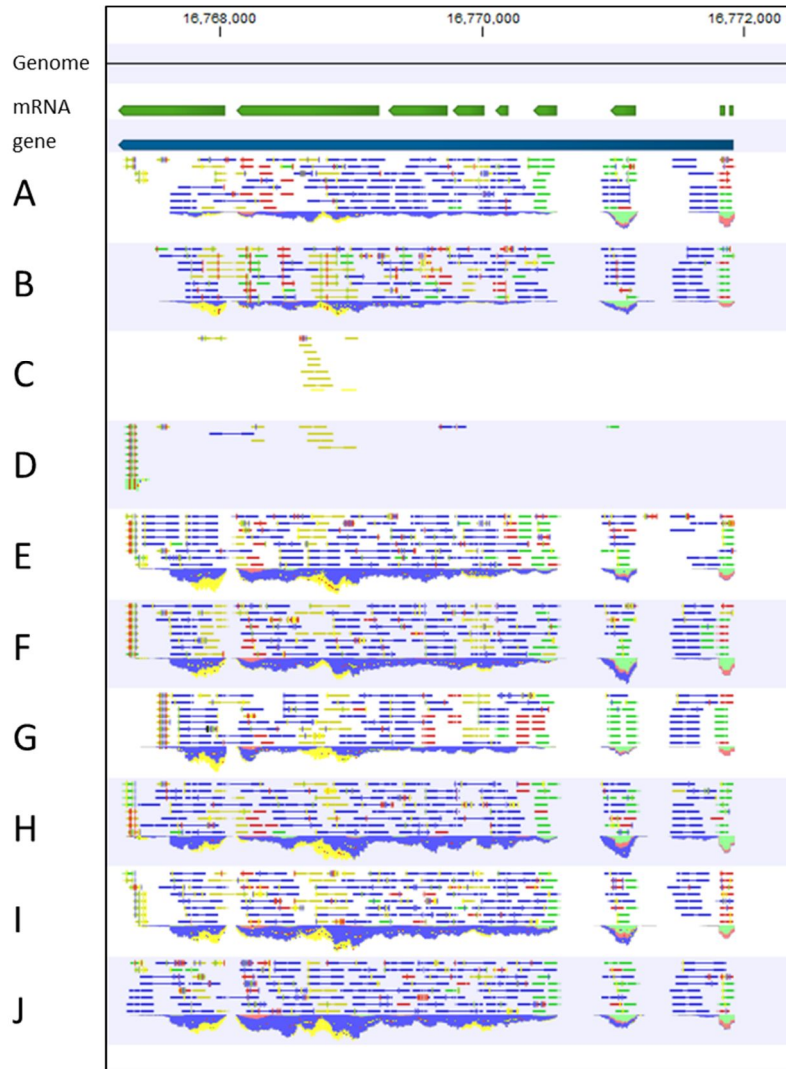


Figure 5. Gene GLYMA17G18930.1 expression among soybean varieties

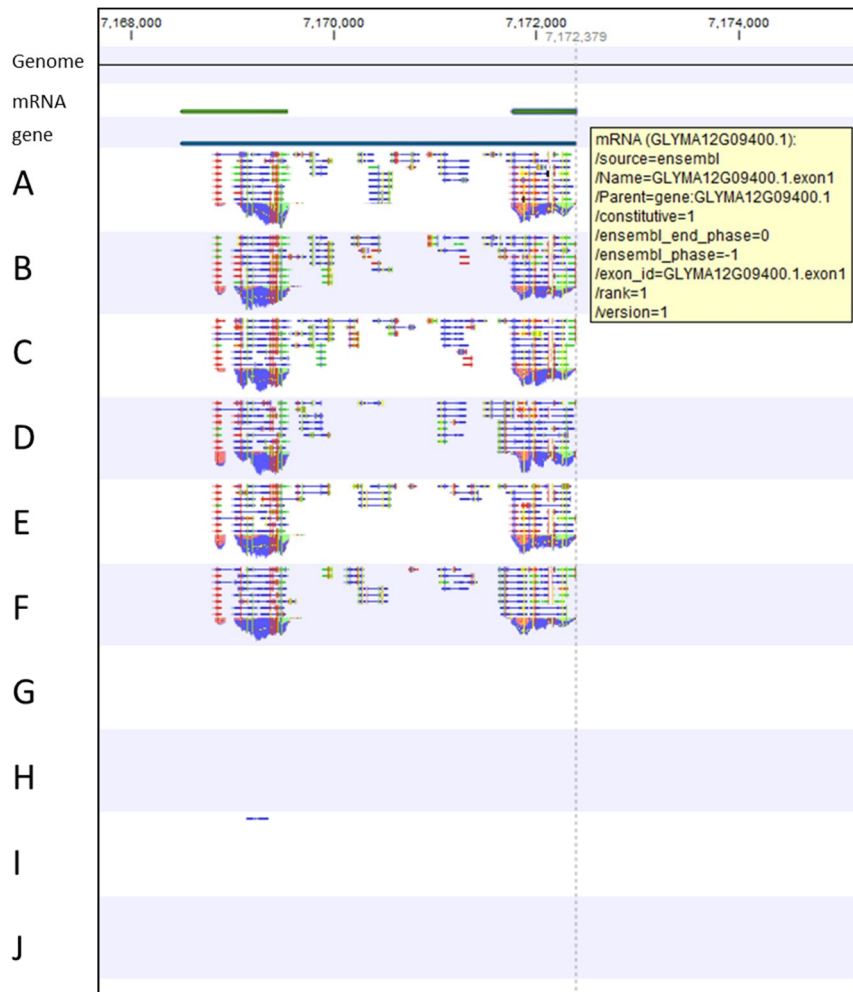


Figure 6. Gene GLYMA12G09400.1 expression among soybean varieties

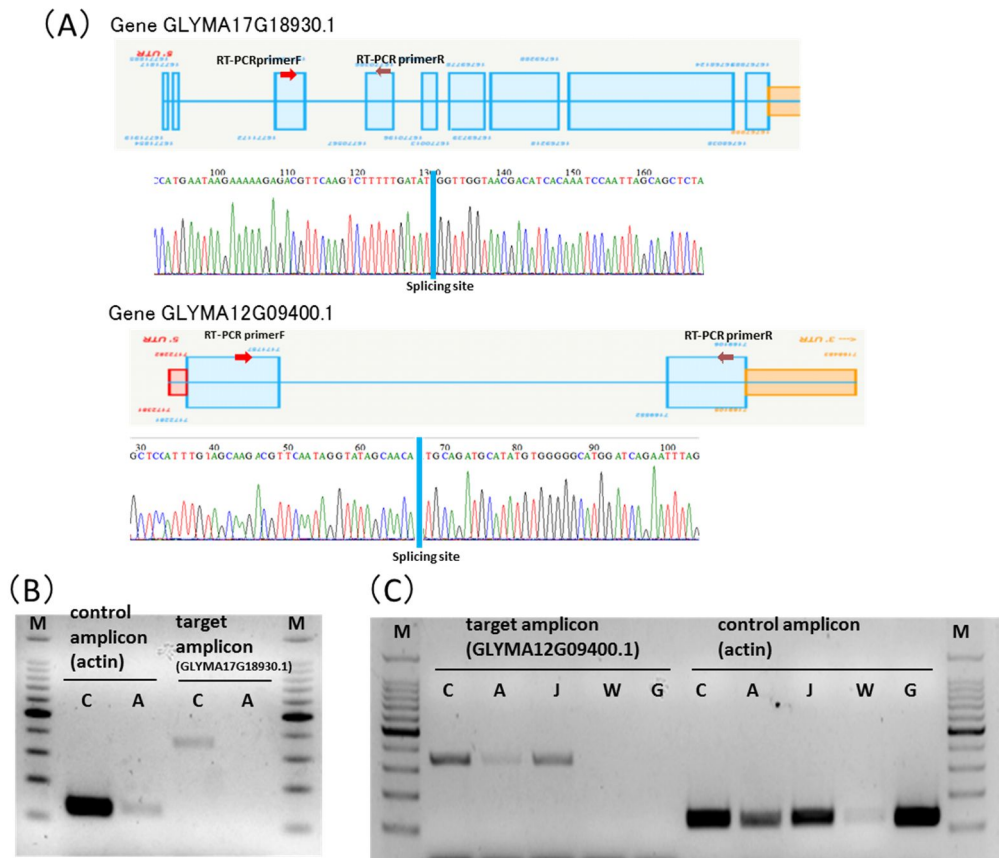


Figure 7. 1% (w/v) agarose gel electrophoresis of the RT-PCR samples

(A) Gene structure of gene GLYMA17G18930.1 and gene GLYMA12G09400.1 in the soybean genome, (B) gene GLYMA17G18930.1 expression analysis, (C) gene GLYMA12G09400.1 expression analysis

C, Canada; A, US; J, Jack; W, Non-GM-type Williams; G, GM-type Williams; M, 100 bp marker

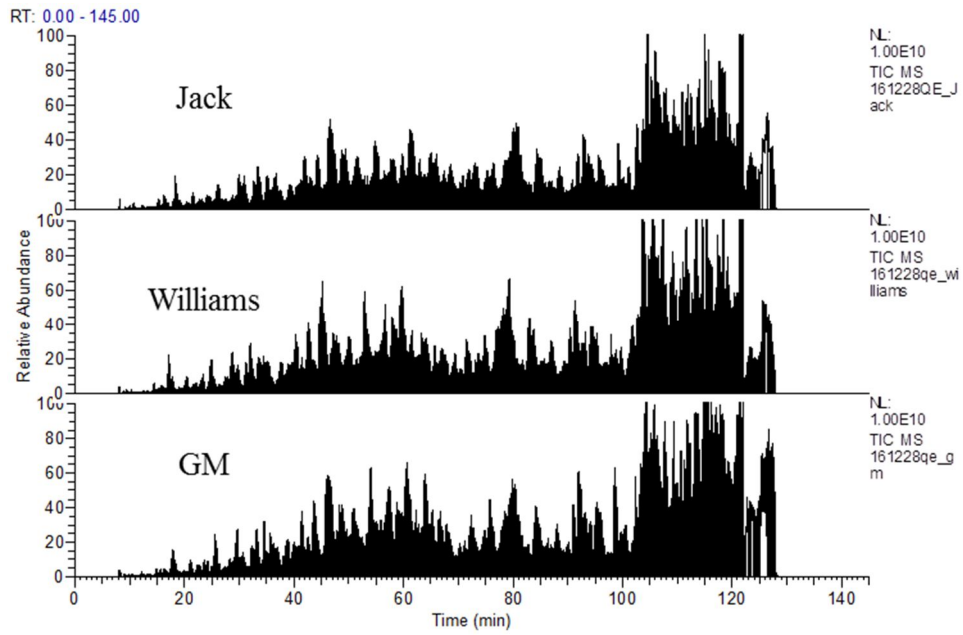


Figure 8. Comparison of TIC chromatography (0-130 min)

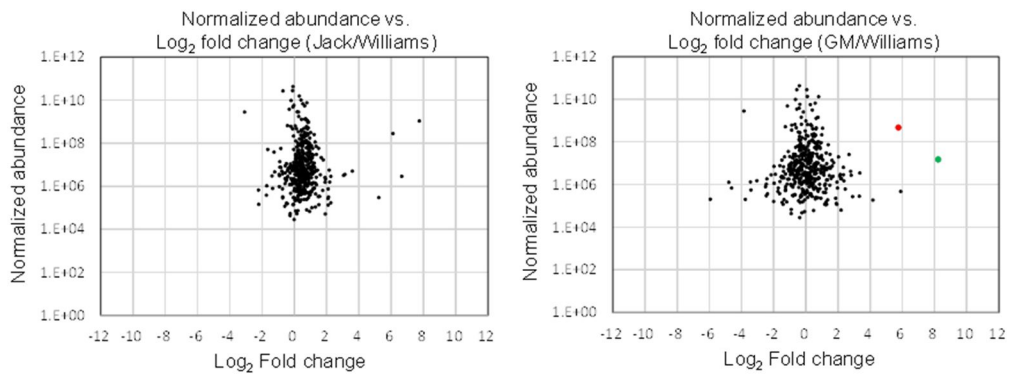
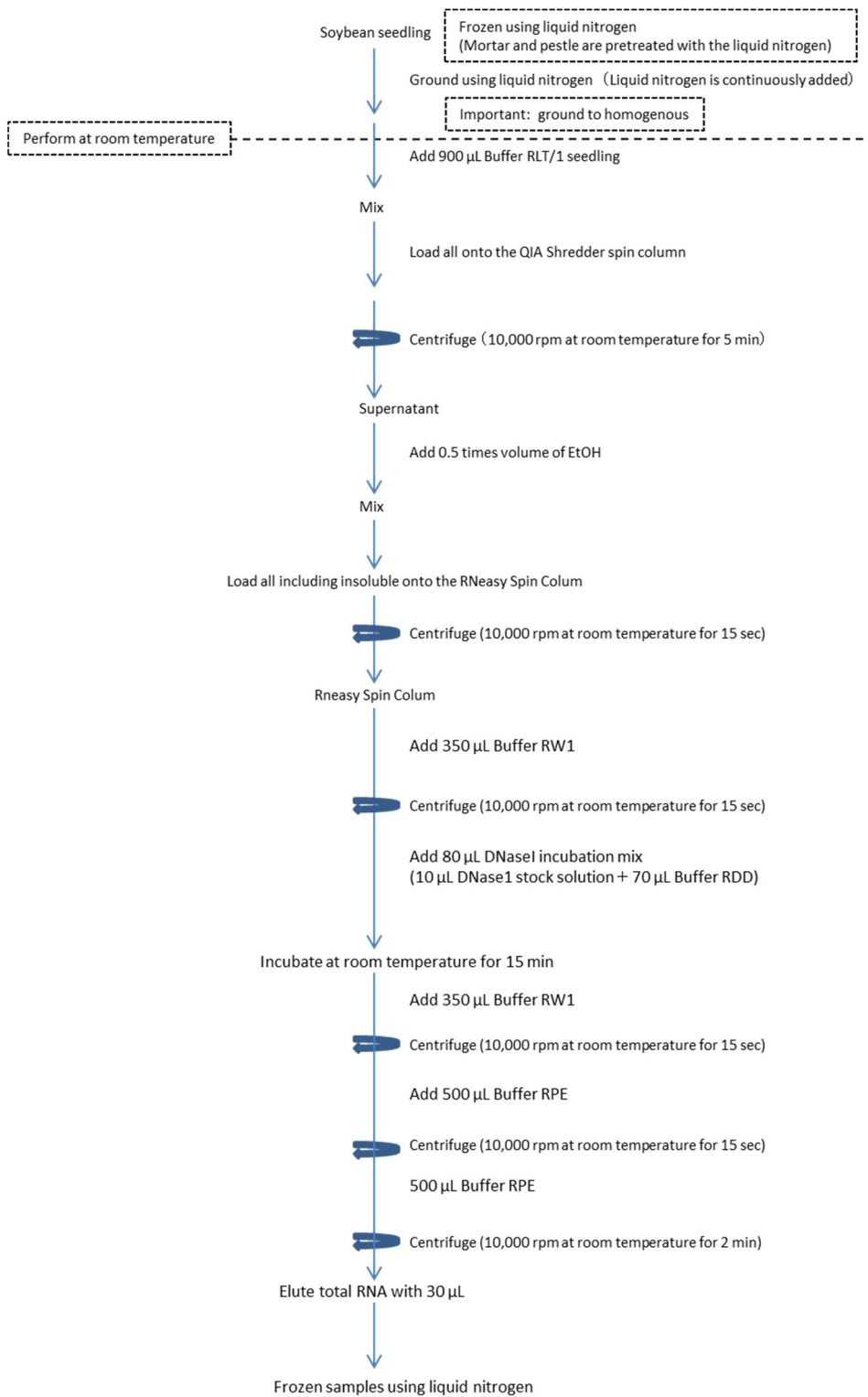
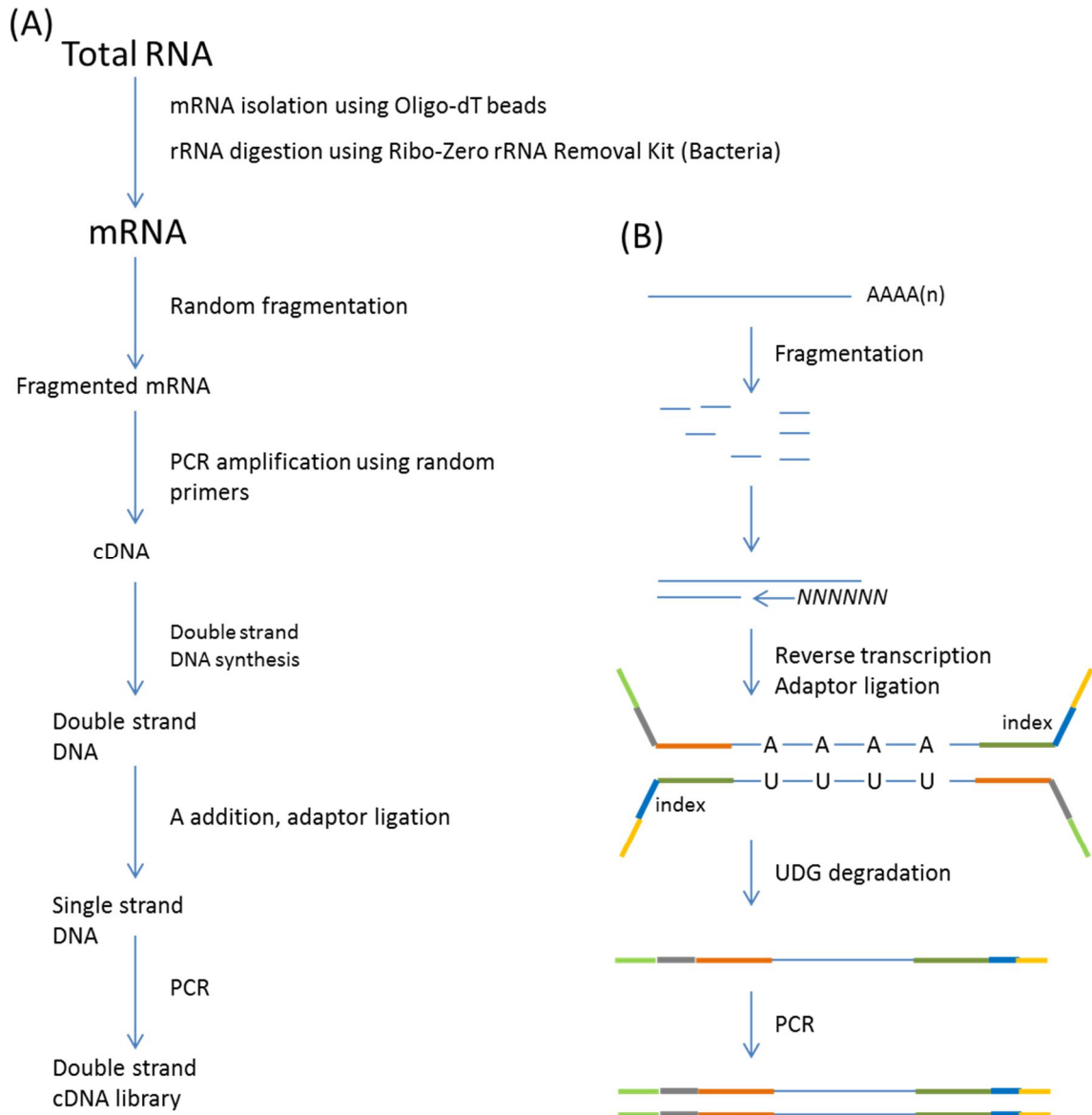


Figure 9 . Scatter plots of normalized abundance and fold change of the proteins eGFP and SYNC1 are marked with red and green points, respectively.



Scheme1. Purification scheme for the total RNAs



Scheme 2. RNA-Seq sample preparation

(A) Experimental steps for double strand cDNA library preparation

(B) Schematic diagram for the double strand DNA preparation

- ・野生型品種、及び、種子粒間の発現差解析を行う
- ↓
- ・得られる最大上限fold値を閾値に設定する
- ↓
- ・閾値を超えた遺伝子については、RNA-Seq(リファレンスゲノムへのマッピング)データを参照し、差を定性的に確認する
- ↓
- ・検出エラーでないものは、RT-PCRにて、定性的・定量的に確認する

Scheme 3. The flow of the gene expression analysis using RNA-Seq data for germinated non-GM and GM soybeans