

図1 ゲノム編集による意図しない変化

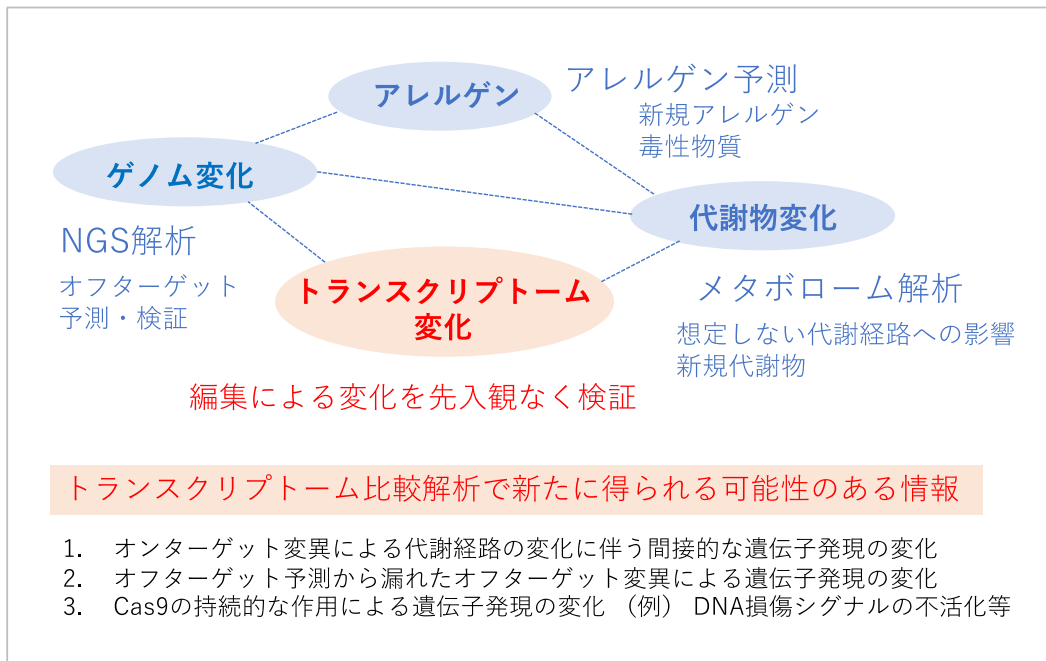


図2 遺伝子発現解析スキーム

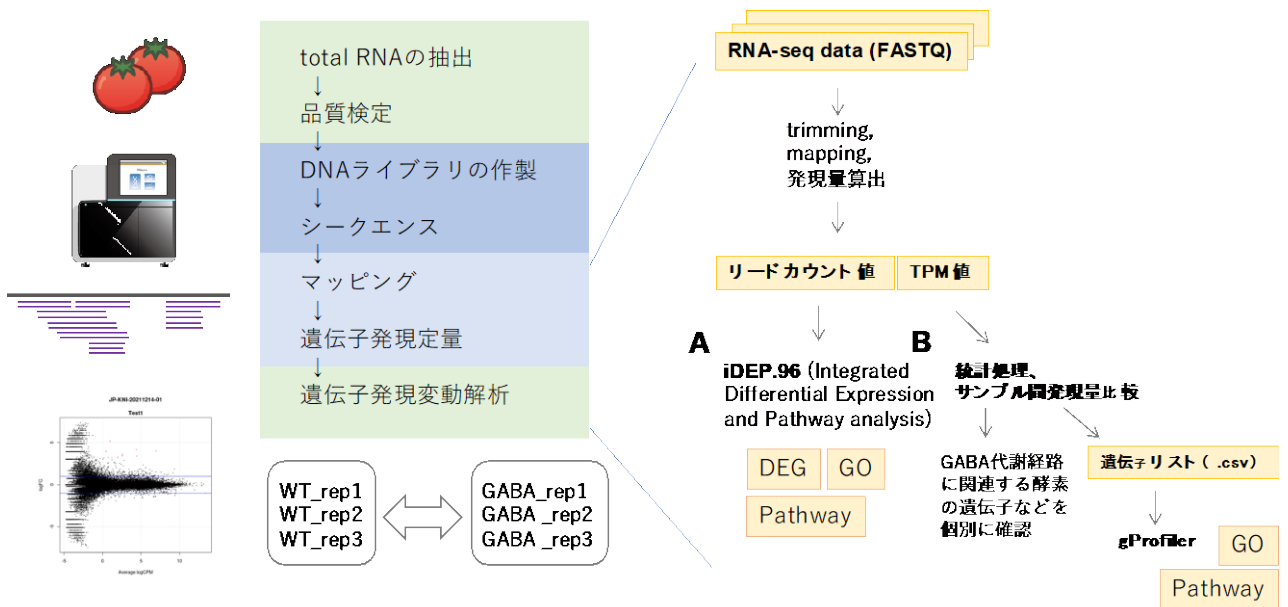


図3 iDEP workflow
 (<http://bioinformatics.sdstate.edu/idep96/>)

iDEP: Integrated Differential Expression and Pathway analysis

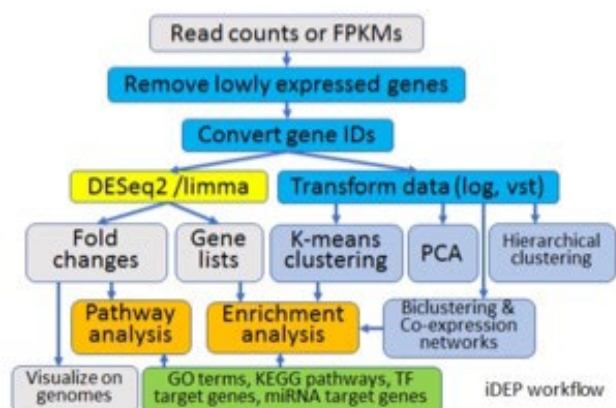


図4 total RNA品質検定結果

品質検定結果

サンプル ID	残量 (ul)	Agilent TapeStation		
		濃度 (ng/ul)	総量 (ng)	RIN [®]
PR3188_01.a	29.0	22.9	664.1	6.5
PR3188_02.a	28.0	13.0	364.0	5.8
PR3188_03.a	28.0	16.5	460.6	7.5
PR3188_04.a	28.0	18.3	512.4	6.3
PR3188_05.a	28.0	16.1	450.8	7.5
PR3188_06.a	28.0	16.0	446.6	6.2

サンプル情報

サンプル ID	依頼書記載名称
PR3188_01.a	wt1
PR3188_02.a	mt1
PR3188_03.a	wt2
PR3188_04.a	mt2
PR3188_05.a	wt3
PR3188_06.a	mt3

*各数値は小数点以下 1 桁で表記しています。未検出の場合は N.D. と表記しています。

Agilent TapeStation 分析結果

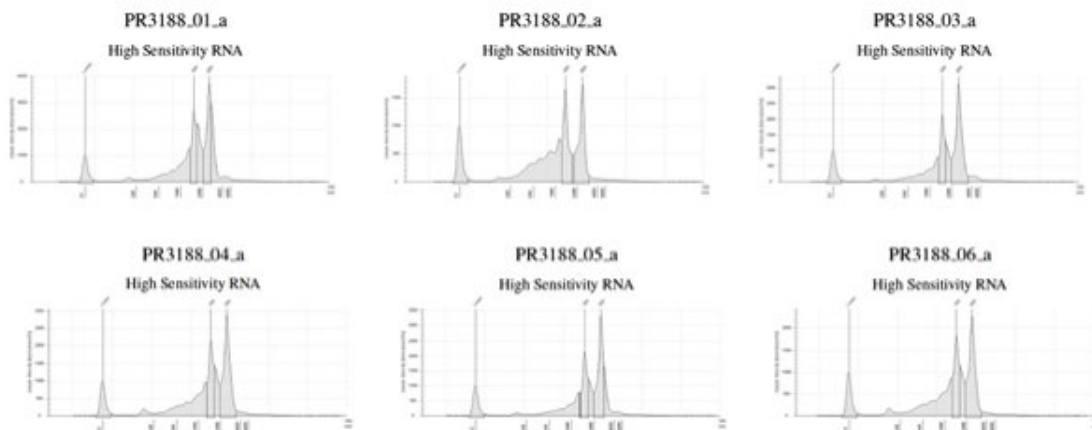


図5 発現変動遺伝子のプロット (GABA/WT)

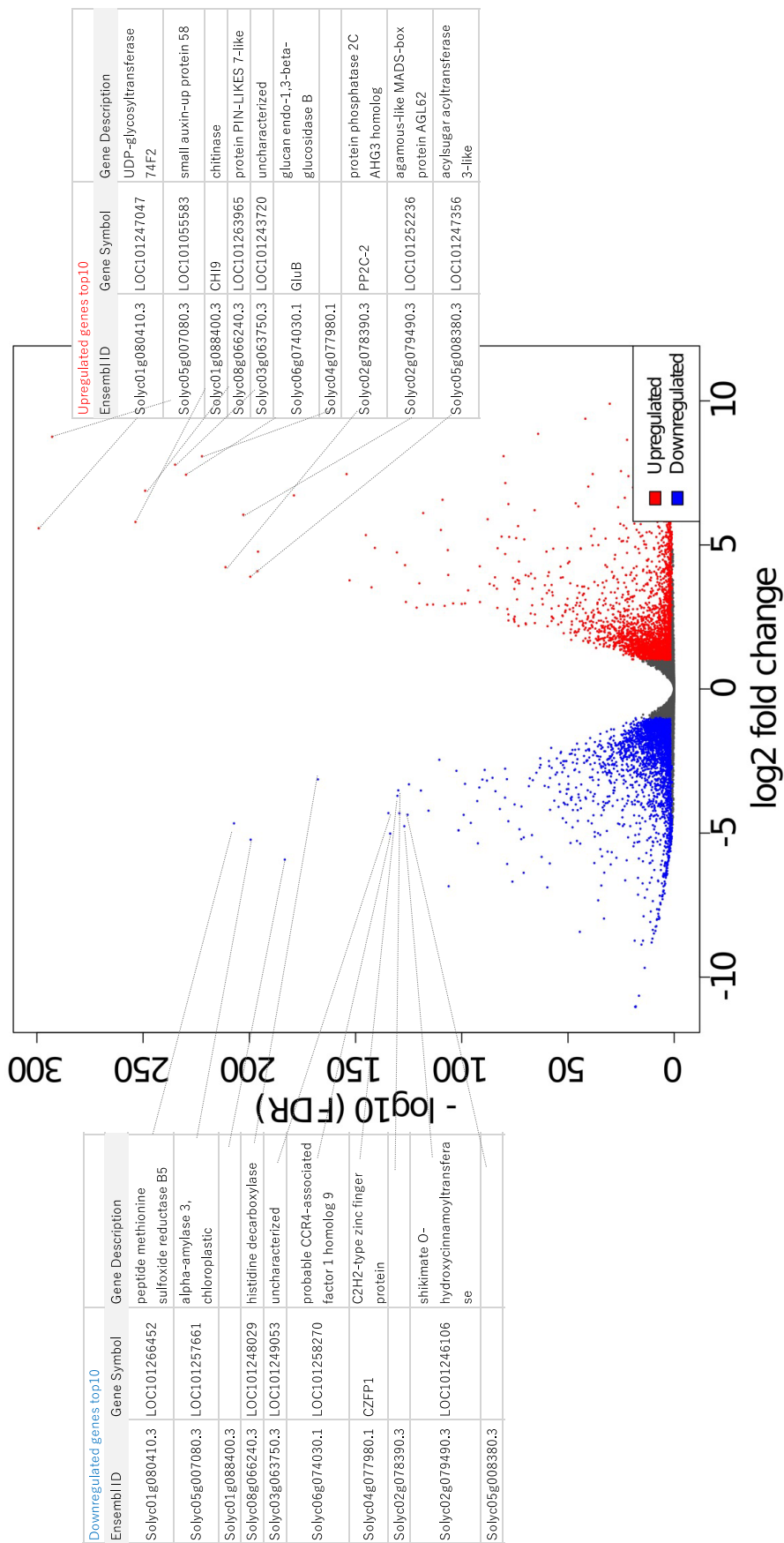
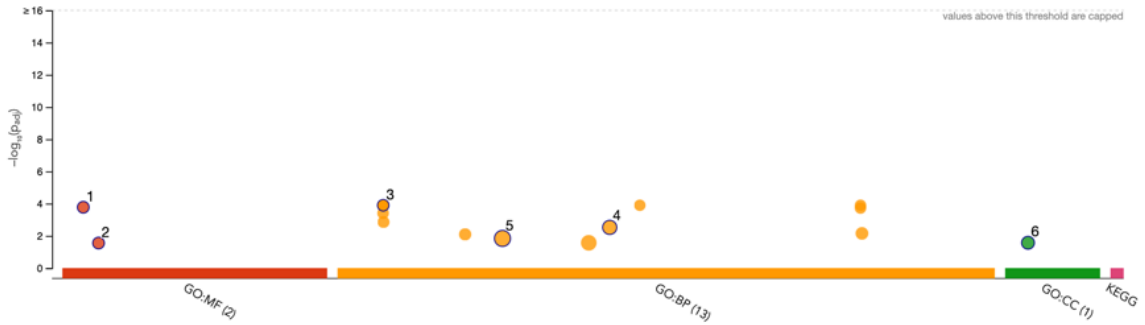


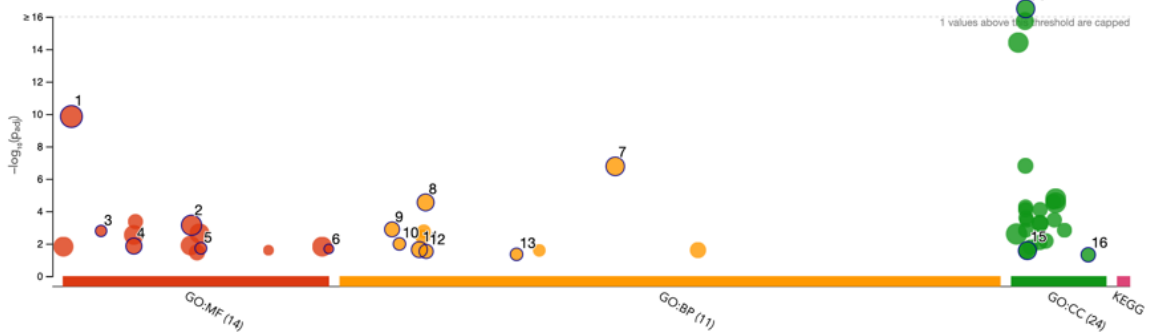
図6 gProfilerによるGO解析結果

Upregulated



ID	Source	Term ID	Term Name	Padj (query_1)
1	GO:MF	GO:0004568	chitinase activity	1.672×10 ⁻⁴
2	GO:MF	GO:0008061	chitin binding	2.795×10 ⁻²
3	GO:BP	GO:0006026	aminoglycan catabolic process	1.260×10 ⁻⁴
4	GO:BP	GO:0044036	cell wall macromolecule metabolic process	2.970×10 ⁻³
5	GO:BP	GO:0022613	ribonucleoprotein complex biogenesis	1.443×10 ⁻²
6	GO:CC	GO:0030684	preribosome	2.678×10 ⁻²

Downregulated



ID	Source	Term ID	Term Name	Padj (query_1)
1	GO:MF	GO:0003824	catalytic activity	1.413×10 ⁻¹⁰
2	GO:MF	GO:0036094	small molecule binding	7.243×10 ⁻⁴
3	GO:MF	GO:0008235	metalloexopeptidase activity	1.633×10 ⁻³
4	GO:MF	GO:0016791	phosphatase activity	1.397×10 ⁻²
5	GO:MF	GO:0043621	protein self-association	1.923×10 ⁻²
6	GO:MF	GO:2001070	starch binding	2.095×10 ⁻²
7	GO:BP	GO:0044281	small molecule metabolic process	1.689×10 ⁻⁷
8	GO:BP	GO:0009628	response to abiotic stimulus	2.834×10 ⁻⁵
9	GO:BP	GO:0006399	tRNA metabolic process	1.306×10 ⁻³
10	GO:BP	GO:0006779	porphyrin-containing compound biosynthetic p...	1.036×10 ⁻²
11	GO:BP	GO:0009117	nucleotide metabolic process	2.318×10 ⁻²
12	GO:BP	GO:0009657	plastid organization	2.995×10 ⁻²
13	GO:BP	GO:0031146	SCF-dependent proteasomal ubiquitin-depend...	4.519×10 ⁻²
14	GO:CC	GO:0009536	plastid	5.904×10 ⁻¹⁸
15	GO:CC	GO:0012505	endomembrane system	2.693×10 ⁻²
16	GO:CC	GO:0098791	Golgi apparatus subcompartment	4.825×10 ⁻²

図7 SIGAD3 read mapping



図8 高等動物におけるGABA代謝経路 (参考文献2より抜粋)

GABAトマトは、ゲノム編集によりグルタミン酸脱炭酸酵素(GAD)遺伝子の自己阻害領域の除去を行うことでGADの活性を上昇させGABA蓄積量を向上させている。

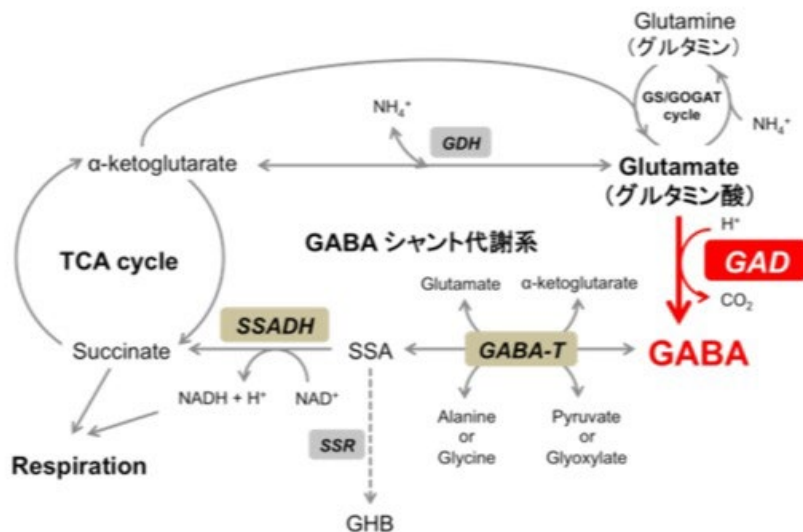


表1 マッピング結果

Sample ID	Total input reads	Mapped reads	Mapped reads (%)	Mapped reads R1	Mapped reads R1 (%)	Mapped reads R2	Mapped reads R2 (%)	Unmapped reads	Unmapped reads (%)	Singleton reads (itself mapped; mate unmapped)	Singleton reads (itself mapped; mate unmapped) (%)
wt1	46082366	45903657	99.61	22960132	99.65	22943525	99.58	178709	0.39	45361	0.1
mt1	47732748	47398604	99.3	23705867	99.33	23692737	99.27	334144	0.7	65088	0.14
wt2	45115786	44944938	99.62	22478825	99.65	22466113	99.59	170848	0.38	36536	0.08
mt2	96484460	95702762	99.19	47862501	99.21	47840261	99.17	781698	0.81	138076	0.14
wt3	48528112	48334153	99.6	24173643	99.63	24160510	99.57	193959	0.4	32997	0.07
mt3	52680576	52263148	99.21	26141942	99.25	26121206	99.17	417428	0.79	75776	0.14

Paired reads (itself & mate mapped)	Paired reads (itself & mate mapped) (%)	Properly paired reads	Properly paired reads (%)	Not properly paired reads (discordant)	Not properly paired reads (discordant) (%)	Reads with MAPQ [10:inf]	Reads with MAPQ [10:inf] (%)	Covered bases (%)	Total alignments	Insert length: mean	Insert length: median	Insert length: standard deviation
45858296	99.51	44992084	97.63	866212	1.88	42790760	92.86	6.77	51275540	192	192	81.66
47333516	99.16	46463430	97.34	870086	1.82	42427878	88.89	6.53	57886941	173	173	74.83
44908402	99.54	44228140	98.03	680262	1.51	42414296	94.01	7.51	49890905	205	205	86.42
95564686	99.05	93782610	97.2	1782076	1.85	86095587	89.23	8.14	115471297	173	173	71.04
48301156	99.53	47775146	98.45	526010	1.08	45878385	94.54	6.96	54556178	158	158	62.16
52187372	99.06	51340186	97.46	847186	1.61	47345311	89.87	7.4	62631102	136	136	51.92

表2 iDEPによるGO解析結果

GO: Biological Process

Enriched pathways in DEGs for the selected comparison:

Direction	adj.Pval	nGenes	Pathways
Down regulated	1.6e-06	178	Carbohydrate metabolic process
	8.6e-03	46	Carbohydrate catabolic process
Up regulated	2.0e-09	94	Ribonucleoprotein complex biogenesis
	7.2e-08	166	Peptide metabolic process
	1.4e-07	75	Ribosome biogenesis
	3.6e-07	149	Translation
	4.4e-07	149	Peptide biosynthetic process
	1.1e-06	175	Cellular amide metabolic process
	2.5e-06	153	Amide biosynthetic process
	3.7e-06	44	Ribonucleoprotein complex assembly
	4.6e-06	44	Ribonucleoprotein complex subunit organization
	5.7e-06	33	Ribosomal large subunit biogenesis
	1.6e-05	166	Cellular component biogenesis
Up regulated	3.2e-04	26	Ribosome assembly
	4.4e-04	23	Cytoplasmic translation
	6.2e-04	104	Protein-containing complex subunit organization
	1.5e-03	9	Aminoglycan catabolic process

GO: Molecular Function

Enriched pathways in DEGs for the selected comparison:

Direction	adj.Pval	nGenes	Pathways
Down regulated	2.8e-03	69	Hydrolase activity, hydrolyzing O-glycosyl compounds
	2.8e-03	49	Vitamin binding
	2.8e-03	45	Carbohydrate binding
	2.8e-03	524	Small molecule binding
	2.8e-03	37	ADP binding
	3.4e-03	74	Hydrolase activity, acting on glycosyl bonds
	6.6e-03	231	Kinase activity
	6.6e-03	491	Anion binding
	6.6e-03	34	Pyridoxal phosphate binding
	6.6e-03	34	Vitamin B6 binding
Up regulated	7.9e-03	264	Transferase activity, transferring phosphorus-containing groups
	7.9e-03	215	Phosphotransferase activity, alcohol group as acceptor
	5.3e-13	124	Structural constituent of ribosome
	9.5e-12	138	Structural molecule activity
	2.0e-04	11	Chitin binding
7.7e-03	9	Chitinase activity	

GO: Cellular Component

Enriched pathways in DEGs for the selected comparison:

Direction	adj.Pval	nGenes	Pathways	
Down regulated	1.0e-06	218	Chloroplast	
	1.0e-06	223	Plastid	
	1.4e-03	61	Photosynthetic membrane	
	1.4e-03	54	Thylakoid membrane	
	2.4e-03	60	Plastid stroma	
	2.4e-03	54	Chloroplast thylakoid	
	2.4e-03	57	Chloroplast stroma	
	2.4e-03	54	Plastid thylakoid	
	3.2e-03	71	Thylakoid	
	3.6e-03	47	Chloroplast thylakoid membrane	
	3.6e-03	57	Plastid membrane	
	3.6e-03	47	Plastid thylakoid membrane	
	5.4e-03	77	Plastid envelope	
	Up regulated	2.1e-19	83	Cytosolic ribosome
		9.4e-17	92	Ribosomal subunit
2.8e-15		56	Cytosolic large ribosomal subunit	
2.8e-15		140	Ribonucleoprotein complex	
3.7e-15		129	Ribosome	
1.8e-13		62	Large ribosomal subunit	
5.8e-12		228	Non-membrane-bounded organelle	
5.8e-12		228	Intracellular non-membrane-bounded organelle	
1.5e-05		57	Extracellular region	
1.5e-05		27	Cytosolic small ribosomal subunit	
Up regulated	2.9e-05	167	Cytosol	
	8.1e-05	21	Preribosome	
	3.2e-04	30	Small ribosomal subunit	
	2.0e-03	22	DNA packaging complex	
	6.3e-03	12	Mitochondrial outer membrane	

GO: KEGG

Enriched pathways in DEGs for the selected comparison:

Direction	adj.Pval	nGenes	Pathways
Down regulated	8.4e-03	23	Starch and sucrose metabolism
	1.0e-02	14	Photosynthesis
Up regulated	2.2e-15	78	Ribosome

表3 発現変動遺伝子リスト (一部)

	A	B	C	D	E	F	G	N	O	P	Q	R	S	T	
1	TPM値							log2TPM_FoldChange		Variance					
2	Gene ID	wt_rep1	gaba_rep1	wt_rep2	gaba_rep2	wt_rep3	gaba_rep3	FoldChange	ABS	wt	gaba	t-test	p-value	t-test	
								e				p-v	rank	q-v	
3	ENSRNA050030304	0	113.437	0	64.827	0	110.85	16.511379	16.51138	0	0.208613	0.0003	505	0.0095	
4	Solyc04g012140.1	29.806	0	61.834	0	88.26	0	-15.73633	15.73633	0.637435	0	0.0009	1116	0.0095	
5	Solyc04g081655.1	30.867	0	66.012	0	61.593	0	-15.6116	15.6116	0.367662	0	0.0005	792	0.0095	
6	Solyc01g091590.3	65.582	0	106.792	0.058	92.052	0	-14.43768	14.43768	0.129997	11.53516	0.0171	5938	0.0095	
7	Solyc11g032190.1	13.351	0	58.277	0	13.431	0	-14.41627	14.41627	1.500395	0	0.0024	2198	0.0095	
8	Solyc02g088595.1	22.325	0	16.301	0	19.186	0	-14.22235	14.22235	0.051478	0	8E-05	214	0.0095	
9	Solyc09g061793.1	16.087	0	19.492	0	21.593	0	-14.20757	14.20757	0.046474	0	8E-05	201	0.0095	
10	ENSRNA050029545	0	13.222	0	8.974	0	41.72	14.056981	14.05698	0	1.329127	0.0022	2108	0.0099	
11	Solyc05g006145.1	14.205	0	19.523	0	15.61	0	-13.99248	13.99248	0.055515	0	9E-05	236	0.0099	
12	Solyc09g082480.2	0	7.577	0	18.362	0	26.041	13.906889	13.90689	0	0.842725	0.0014	1590	0.0101	
13	ENSRNA050030085	0	9.562	0	8.621	0	42.789	13.894013	13.89401	0	1.672833	0.0029	2424	0.0101	
14	ENSRNA050029941	0	20.187	0	12.66	0	9.995	13.738816	13.73882	0	0.26628	0.0005	747	0.0101	
15	Solyc09g066400.2	0	20.6	0	10.08	0	8.804	13.577962	13.57796	0	0.434183	0.0008	1051	0.0101	
16	ENSRNA050029545	0	19.832	0	2.991	0	29.8	13.561861	13.56186	0	3.131344	0.0056	3486	0.0101	
18	Solyc03g019690.1	0.123	44.369	0	59.489	0	63.101	13.429619	13.42962	16.12028	0.074063	0.0281	7272	0.0101	
19	Solyc02g087215.1	0	6.68	0	14.224	0	12.47	13.369426	13.36943	0	0.339263	0.0006	918	0.0101	
20	Solyc01g005290.3	15.934	0	8.645	0	7.785	0	-13.32147	13.32147	0.311422	0	0.0006	876	0.0101	
21	Solyc03g093550.1	43.78	0	35.463	0.047	32.017	0	-13.30457	13.30457	0.052986	10.39727	0.0186	6170	0.0101	
22	ENSRNA050029561	0	8.13	0	3.673	0	29.23	13.222522	13.22252	0	2.278952	0.0043	3042	0.0101	
23	Solyc01g095500.3	0	4.341	0	12.609	0	15.586	13.211493	13.21149	0	0.976562	0.0019	1849	0.0101	
24	Solyc06g065890.1	0	9.562	0	4.31	0	17.116	13.120066	13.12007	0	0.997349	0.0019	1900	0.0101	
25	Solyc05g054650.1	0	3.712	0	10.767	0	13.585	12.994222	12.99422	0	0.995739	0.002	1922	0.0101	
27	Solyc10g076380.2	0	10.928	0	4.58	0	9.523	12.931558	12.93156	0	0.454656	0.0009	1159	0.0101	
28	Solyc02g085510.1	9.933	0	14.384	0	3.209	0	-12.91293	12.91293	1.270681	0	0.0025	2270	0.0101	
29	Solyc01g011093.1	6.321	0	8.597	0	7.878	0	-12.87992	12.87992	0.05226	0	0.0001	258	0.0101	
31	Solyc09g060160.2	8.164	0	5.108	0	8.346	0	-12.78037	12.78037	0.160019	0	0.0003	596	0.0104	
32	Solyc01g095995.1	6.774	0	5.474	0	7.529	0	-12.67436	12.67436	0.054852	0	0.0001	272	0.0104	
33	Solyc06g083130.3	0	10.081	0	4.796	0	5.495	12.650526	12.65053	0	0.325499	0.0007	961	0.0105	
34	Solyc09g065770.1	0	5.194	0	7.417	0	5.667	12.556116	12.55612	0	0.071766	0.0002	337	0.0105	
35	Solyc03g117130.3	0	6.425	0	4.937	0	6.781	12.54897	12.54897	0	0.060002	0.0001	299	0.0105	
36	Solyc10g050510.1	0	4.076	0	5.527	0	7.339	12.422466	12.42247	0	0.179968	0.0004	669	0.0144	
37	Solyc08g067640.1	0	9.982	0	3.181	0	5.013	12.404241	12.40424	0	0.689736	0.0015	1615	0.0195	
38	Solyc01g010310.3	0	5.778	0	7.986	0	3.267	12.378078	12.37808	0	0.426075	0.0009	1175	0.0195	
41	Solyc09g014390.1	0	8.224	0	3.117	0	4.949	12.295139	12.29514	0	0.489937	0.0011	1306	0.0113	
42	ENSRNA050030331	0	9.25	0	2.426	0	5.149	12.250236	12.25024	0	0.936459	0.0021	2008	0.0113	
44	Solyc02g062770.2	0	10.079	0	2.512	0	3.101	12.064462	12.06446	0	1.16652	0.0027	2333	0.0113	
45	Solyc10g080840.1	15.103	0	19.94	0	18.625	0.082	-11.99203	11.99203	0.043628	13.54704	0.0297	7440	0.0113	
46	Solyc06g062520.1	6.832	0	3.137	0	3.004	0	-11.96903	11.96903	0.444815	0	0.001	1274	0.0113	
47	Solyc06g074750.1	6.444	0	3.088	0	3.077	0	-11.94489	11.94489	0.377098	0	0.0009	1138	0.0113	
49	Solyc07g054550.1	0	4.475	0	8.573	0	1.464	11.903477	11.90348	0	1.662243	0.0039	2860	0.0113	
50	Solyc07g038137.1	2.915	0	3.554	0	5.126	0	-11.87644	11.87644	0.170596	0	0.0004	684	0.0113	
51	Solyc09g057520.2	2.42	0	3.727	0	4.326	0	-11.72824	11.72824	0.189323	0	0.0005	727	0.0113	
52	Solyc02g077570.2	0	5.8	0	2.576	0	2.518	11.710733	11.71073	0	0.46995	0.0011	1355	0.0113	
53	Solyc06g072193.1	2.369	0	3.985	0	3.862	0	-11.69563	11.69563	0.176903	0	0.0004	705	0.0113	
54	Solyc03g098125.1	3.95	0	3.177	0	2.713	0	-11.6627	11.6627	0.074008	0	0.0002	397	0.0113	
55	Solyc03g020050.3	0	1.907	0	3.435	0	5.195	11.662515	11.66252	0	0.527544	0.0013	1469	0.0218	
57	Solyc03g095860.3	8.897	0	8.978	0.027	10.5	0	-11.60083	11.60083	0.018052	7.703554	0.0184	6137	0.0176	
58	Solyc05g014830.2	0	1.896	0	3.599	0	4.228	11.583129	11.58313	0	0.37433	0.0009	1180	0.0176	
59	Solyc07g051990.2	2.287	0	2.453	0	4.832	0	-11.55316	11.55316	0.355025	0	0.0009	1142	0.0176	
60	Solyc03g020035.1	3.623	0	1.958	0	3.758	0	-11.54513	11.54513	0.279069	0	0.0007	982	0.0176	
61	Solyc08g062515.1	7.881	0	10.681	0.025	7.918	0	-11.52597	11.52597	0.063136	7.364711	0.0173	5973	0.0344	
62	Solyc06g061110.2	0	7.746	0	1.448	0	2.078	11.480646	11.48065	0	1.620374	0.0041	2941	0.0344	
65	Solyc08g082490.2	2.148	0	3.193	0	2.755	0	-11.37965	11.37965	0.083489	0	0.0002	448	0.0076	
66	Solyc09g018550.1	2.71	0	2.842	0	2.433	0	-11.37564	11.37564	0.013183	0	3E-05	102	0.0076	
67	Solyc07g006670.1	0	6.394	0	1.508	0	1.83	11.346841	11.34684	0	1.27896	0.0033	2618	0.0076	
68	Solyc03g095870.2	2.627	0	2.348	0	2.787	0	-11.3342	11.3342	0.015765	0	4E-05	116	0.0076	
69	Solyc01g009700.2	1.837	0	3.281	0	2.778	0	-11.32154	11.32154	0.185494	0	0.0005	762	0.0076	
70	Solyc05g010367.1	2.563	0	1.63	0	3.775	0	-11.29279	11.29279	0.367431	0	0.001	1209	0.0076	
72	Solyc09g072655.1	2.469	0	2.675	0	2.202	0	-11.2538	11.2538	0.019889	0	5E-05	140	0.0076	
73	ENSRNA050030350	0	4.18	0	1.867	0	1.844	11.248772	11.24877	0	0.457387	0.0012	1396	0.0076	
74	Solyc03g063460.1	0	1.895	0	0.847	0	8.372	11.216064	11.21606	0	2.808811	0.0074	3986	0.0076	
76	Solyc08g067360.3	0	0.777	0	3.689	0	3.993	11.138883	11.13888	0	1.771022	0.0047	3189	0.0076	
77	ENSRNA050029620	0	2.357	0	2.095	0	2.123	11.096446	11.09645	0	0.008663	2E-05	77	0.0076	

表4 GAD遺伝子の発現変動

EnsemblID	GeneID	Symbol	Aliases	description	TPM値						log2TPM_FoldChange			Variance		p-value rank	t-test p-value	t-test q-value
					wt_rep1	gaba_rep1	wt_rep2	gaba_rep2	wt_rep3	gaba_rep3	FoldChange	ABS	wt	gaba				
Solyc01g050000.3	100147723	GAD3	LeGAD3, SIGAD3	glutamate decarboxylase isoform3	0.333	0.334	1.079	0.722	1.129	0	-3.57225261	3.57225261	0.99381152	26.966626	0.3554154	16660		
Solyc05g054050.3	101251185	LOC101251185	SIGAD4	glutamate decarboxylase-like	0	0	0	0	0	0.069	2.04309434	2.04309434	0	12.5227034	0.4226497	19864		
Solyc11g011920.2	100147722	GAD2	LeGAD2	glutamate decarboxylase isoform2	3.116	0.205	6.311	2.09	4.221	2.448	-2.0996933	2.09969329	0.26073984	3.99771095	0.205406	14584		
Solyc03g098240.3	544313	LOC544313	GAD, LeGAD1	glutamate decarboxylase	191.211	65.349	222.49	48.684	220.927	70.643	-1.7953497	1.79534966	0.01521832	0.08024551	0.0030812	2523		0.006431
Solyc04g025530.3	101259235	LOC101259235		glutamate decarboxylase 4	7.653	0	7.389	0.738	6.225	0.671	-6.4785662	6.47856624	0.02540926	29.8407455	0.1762243	13168		

表5 GABA代謝系に関わる遺伝子の発現変動

EnsemblID	GeneID	Symbol	description	TPM値						log2TPM_FoldChange			Variance		p-value rank	t-test p-value	t-test q-value
				wt_rep1	gaba_rep1	wt_rep2	gaba_rep2	wt_rep3	gaba_rep3	FoldChange	ABS	wt	gaba				
Solyc09g090700.1	100147726	SSADH	Succinic semialdehyde dehydrogenase	84.863	42.671	99.919	30.189	94.095	44.535	-1.26590903	1.26590903	0.01420315	0.09460575	0.01084191	4873		0.03642051
Solyc03g094010.3	101246116	LOC101246116	glutamate dehydrogenase	2.619	0.977	2.167	2.031	2.89	2.813	-0.51802369	0.51802369	0.04453302	0.60973497	0.3701088	16825		
Solyc12g006470.2	543874	GABA-TP2	gamma aminobutyrate transaminase 2	0.302	0.043	0	0.019	0	0	0.512728577	0.51272858	22.6499724	8.29639505	0.88246467	23630		
Solyc12g006450.2	544258	GABA-TP3	gamma-aminobutyrate transaminase subunit precursor isozyme 3	21.02	1	40.991	0.866	41.31	1.651	-4.8665748	4.8665748	0.31308473	0.23841537	0.00037936	661		0.01423556
Solyc07g043310.3	543873	GABA-TP1	gamma-aminobutyrate transaminase subunit precursor isozyme 1	70.52	307.092	93.803	226.602	85.441	284.238	1.709694533	1.70969453	0.04403772	0.05193769	0.00069136	975		0.01758812