Transcriptome Analysis Combined With Glutathione S-transferase 1 Gene Family Whole-genome Identification Reveals the Mechanism Underlying the Exogenous Application of 5-aminolevulinic Acid to Alleviate Low Temperature Stress in *Solanum Lycopersicum* Seedling

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Abstract

Background: Transcriptome sequencing was conducted to screen out genes that actively respond to exogenous 5-aminolevulinic acid (ALA) induction under low temperature stress. The study used two versions of the tomato genome database to strictly screen and identify tomato glutathione S-transferase (GST) gene families and carried out the related bioinformatics analysis of tomato GST gene family. The expression pattern of SIGST genes induced by exogenous application ALA under low temperature stress was also analysed. Related physiological indicators were determined, and related chemical stains were performed.

Results: RNA sequencing (RNA-seq) results showed that the expression of SIGST gene was different under various treatments, and a large number of SIGST genes widely responded to ALA induction under low temperature stress. Sixty-nine full-length GST genes were identified by screening the two versions of tomato genome databases combined with protein domain analysis. Analysis of gene family phylogenetic tree divided the tomato GST gene family into eight subfamilies. Tandem replication of genes is one of the driving forces for the evolution of tomato GST gene family, and a large number of cis-acting elements are related to stress resistance on the promoter of the GST gene family. Exogenous ALA application under low temperature stress induces a broad response of tomato leaf SIGST gene (qRT-PCR verification), increases GST activity and decreases reactive oxygen species (ROS) accumulation.

Conclusions: RNA sequencing results revealed that a large number of tomato GST genes are differentially expressed, and Sixty-nine GSTs are identified in the tomato genome. Tandem replication of genes is the driving force for 68 the evolution of tomato GST family, and the promoter contains a large number of cis-acting elements related to stress resistance. Test results show that exogenous ALA induces the expression of SIGST genes under low temperature stress, thereby increasing GST activity to eliminate the ROS produced under low temperature stress and increase the tomato tolerance.

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Tables

Table 1 Summary of RNA-seq data sets

Samples	Clean reads	Clean bases	GC (%)	Q30 (%)	Total reads	Mappable reads	Mapping rate (%)
CK1	8364597146	27936903	43.31	94.58	55873806	49430367	88.47
CK2	8317330184	27789453	43.47	94.69	55578906	48787623	87.78
CK3	7691554432	25699897	43.48	94.93	51399794	45225767	87.99
ALA1	8026187750	26810222	43.32	94.56	53620444	47022467	87.70
ALA2	7404555476	24737565	43.25	94.53	49475130	43805327	88.54
ALA3	7617858308	25449304	43.59	94.33	50898608	43834104	86.12
LT1	6900841340	23049776	42.94	94.41	46099552	40410994	87.66
LT2	7270022498	24279980	42.92	94.24	48559960	42743978	88.02
LT3	11240523464	37570881	44.53	94.26	75141762	58948227	78.45
LTA1	6799325530	22716030	43.22	94.29	45432060	39090523	86.04
LTA2	7234826466	24174036	43.25	94.46	48348072	41217602	85.25
LTA3	6918924098	23158144	43.41	94.48	46316288	39858708	86.06

Table 2 Identified GST genes in tomato with information and location predictions

No.	Gene Name	Gene ID (ITAG 2.40)	Gene ID (ITAG 4.0)	Length (bp)			MW (kDa)	Exons	pI	Protein(aa)	Localization
				Gene	cDNA	CDS					
1	<i>SIGSTF1</i>	Solyc09g074850.2	Solyc09g074850.4.1	2416	1147	814	22.94	6	5.42	200	Cy ^{a,b}
2	<i>SIGSTF2</i>	Solyc02g081340.2	Solyc02g081340.3.1	1359	959	693	26.55	3	5.99	230	Cy ^{a,b}
3	<i>SIGSTF3</i>	Solyc06g009020.2	Solyc06g009020.2.1	2217	1403	642	23.72	3	5.98	213	Cy ^a ,Cp ^b
4	<i>SIGSTF4</i>	Solyc12g094430.1	Solyc12g094430.1.1	2156	666	666	25.07	3	6.85	221	Cy ^a ,Nu ^a ,Cp ^b
5	<i>SIGSTF5</i>	Solyc06g009040.2	Solyc06g009040.4.1	1953	849	639	23.86	3	6.08	212	Cy ^a ,Cp ^b
6	<i>SIGSTT1</i>	Solyc08g080900.2	Solyc08g080900.3.1	4012	1198	753	28.58	7	9.25	250	Cy ^{a,b}
7	<i>SIGSTT2</i>	Solyc08g080910.2	Solyc08g080910.3.1	3707	1369	753	28.69	7	9.21	250	Cy ^{a,b} ,Nu ^b
8	<i>SIGSTT3</i>	Solyc12g056250.1	Solyc12g056250.2.1	2138	711	711	26.55	7	6.06	236	Cy ^a ,Mt ^b
9	<i>SIMGST1</i>	Solyc04g081740.2	Solyc04g081740.3.1	4097	1497	1158	43.48	6	9.14	386	Mt ^a ,Cp ^a ,Cy ^b
10	<i>SIEF1By1</i>	Solyc11g028100.1	Solyc11g028100.2.1	2091	1245	1245	47.33	6	5.94	414	Cp ^a ,Cy ^b
11	<i>SIEF1By2</i>	Solyc06g011280.2	Solyc06g011280.3.1	3077	1675	1242	47.05	8	5.66	413	Cp ^{ab}
12	<i>SIGSTL1</i>	Solyc04g009530.2	Solyc04g009530.4.1	5207	1191	888	33.52	10	6.25	295	Cy ^a ,Cp ^b
13	<i>SIGSTL2</i>	Solyc09g007150.2	Solyc09g007150.3.1	3633	953	717	27.59	10	4.97	238	Cy ^{a,b}
14	<i>SIGSTL3</i>	Solyc10g084400.1	Solyc10g084400.2.1	2340	708	708	27.18	10	5.06	235	Cyt ^{a,b}
15	<i>SIGSTL4</i>	Solyc12g044520.1	Solyc12g044520.2.1	3780	720	720	27.84	10	5.52	239	Cy ^a ,Nu ^b
16	<i>SIGSTL5</i>	Solyc12g044530.1	Solyc12g044530.3.1	3744	789	789	30.36	9	6.84	262	Cy ^a ,Mt ^a ,Cp ^b
17	<i>SIGSTL6</i>	Solyc00g007030.1	Solyc02g038818.1.1	3746	720	720	27.98	10	5.73	239	Cy ^a ,Nu ^b
18	<i>SIGSTL7</i>	Solyc00g007040.1	None	2975	708	708	27.76	8	8.86	235	Cy ^{a,b} ,Mt ^a
19	<i>SIDHAR1</i>	Solyc05g054760.2	Solyc05g054760.4.1	4424	1162	633	23.55	6	6.32	210	Cy ^{a,b}
20	<i>SIDHAR2</i>	Solyc11g011250.1	Solyc11g011250.3.1	5344	807	807	29.85	6	8.59	268	Mt ^a ,Cp ^{ab}
21	<i>SIGSTZ1</i>	Solyc01g091330.2	Solyc01g091330.4.1	6206	1195	855	32.14	10	6.97	284	Cy ^a ,Cp ^b
22	<i>SIGSTZ2</i>	Solyc01g102660.2	Solyc01g102660.4.1	5178	1262	669	25.27	11	5.45	222	Cy ^{a,b}
23	<i>SIGSTU1</i>	Solyc12g036560.1	Solyc12g036560.2.1	333	333	333	12.89	1	6.72	110	Mt ^a ,Nu ^b
24	<i>SIGSTU2</i>	Solyc09g063150.2	Solyc09g063150.3.1	1775	889	663	25.89	2	6.19	220	Cy ^{a,b} ,Cp ^a
25	<i>SIGSTU3</i>	Solyc09g011490.2	Solyc09g011490.3.1	1023	882	660	24.96	2	5.75	219	Cy ^{a,b}
26	<i>SIGSTU4</i>	Solyc09g011500.2	Solyc09g011500.3.1	1584	922	669	25.42	2	5.27	222	Cy ^a ,Cp ^b
27	<i>SIGSTU5</i>	Solyc09g011520.2	Solyc09g011520.3.1	1118	1021	678	26.03	2	6.02	225	Cy ^a ,Cp ^a
28	<i>SIGSTU6</i>	Solyc09g011540.2	Solyc09g011540.2.1	1031	907	669	25.45	2	5.05	222	Cy ^a ,Nu ^b
29	<i>SIGSTU7</i>	Solyc09g011550.2	Solyc09g011550.3.1	1075	864	660	25.36	2	5.45	219	Cy ^{a,b}
30	<i>SIGSTU8</i>	Solyc09g011560.2	Solyc09g011560.3.1	1499	1043	654	25.16	2	5.70	217	Cy ^{a,b}
31	<i>SIGSTU9</i>	Solyc09g011570.2	None	1334	909	633	24.50	2	5.87	210	Cy ^a ,Cp ^b
32	<i>SIGSTU10</i>	Solyc09g011580.2	None	1290	9115	654	25.37	2	5.17	217	Cy ^{a,b}
33	<i>SIGSTU11</i>	Solyc09g011590.2	Solyc09g011590.4.1	1555	903	654	25.11	2	5.30	217	Cy ^{a,b}
34	<i>SIGSTU12</i>	Solyc09g011600.2	Solyc09g011600.3.1	1617	1033	654	25.27	2	5.90	217	Cy ^a ,Nu ^b
35	<i>SIGSTU13</i>	Solyc09g011620.1	Solyc09g011620.2.1	811	648	648	24.76	2	5.39	215	Cy ^a ,Cp ^b
36	<i>SIGSTU14</i>	Solyc09g011630.2	Solyc09g011630.3.1	1278	917	657	24.86	2	5.53	218	Cy ^{a,b}
37	<i>SIGSTU15</i>	Solyc09g011640.2	Solyc09g011640.5.1	1493	891	663	25.27	2	5.82	220	Cy ^a ,Mt ^b
38	<i>SIGSTU16</i>	Solyc01g081260.1	Solyc01g081260.2.1	884	381	381	15.09	2	7.87	126	Cy ^b ,Mt ^a
39	<i>SIGSTU17</i>	Solyc01g081270.2	Solyc01g081270.2.1	1342	1181	672	25.94	2	5.47	223	Cy ^{a,b}
40	<i>SIGSTU18</i>	Solyc09g091130.2	Solyc09g091130.4.1	1006	684	675	26.40	2	7.70	224	Cy ^{a,b} ,Cp ^a
41	<i>SIGSTU19</i>	Solyc12g011300.1	Solyc12g011300.2.1	1128	663	663	25.64	2	5.39	220	Cy ^{a,b}
42	<i>SIGSTU20</i>	Solyc12g011310.1	Solyc12g011310.2.1	1213	660	660	25.62	2	6.91	219	Cy ^a ,Nu ^b
43	<i>SIGSTU21</i>	Solyc12g011320.1	Solyc12g011320.2.1	1187	660	660	25.45	2	5.99	219	Cy ^{a,b}
44	<i>SIGSTU22</i>	Solyc05g006730.2	Solyc05g006730.4.1	1934	954	678	25.55	2	6.32	225	Cy ^a ,Mt ^b
45	<i>SIGSTU23</i>	Solyc05g006740.2	Solyc05g006740.4.1	1911	989	645	24.04	2	5.34	214	Cy ^a ,Cp ^a ,Nu ^b
46	<i>SIGSTU24</i>	Solyc05g006750.2	Solyc05g006750.3.1	1224	979	690	26.31	2	5.10	229	Cy ^{a,b}
47	<i>SIGSTU25</i>	Solyc05g026210.1	Solyc05g026210.2.1	1178	693	693	25.69	2	5.43	230	Cp ^a ,pm ^b
48	<i>SIGSTU26</i>	Solyc12g097080.1	Solyc12g097080.2.1	1153	666	666	25.85	2	6.92	221	Pm ^a ,Cy ^b
49	<i>SIGSTU27</i>	Solyc07g056420.2	Solyc07g056420.4.1	1805	1012	663	25.39	2	5.37	220	Cy ^{a,b}
50	<i>SIGSTU28</i>	Solyc07g056430.2	Solyc07g056430.3.1	1811	1432	1075	25.71	2	5.60	222	Cy ^{a,b}
51	<i>SIGSTU29</i>	Solyc07g056440.2	Solyc07g056440.3.1	1710	757	420	16.35	2	5.31	139	Cy ^{a,b}
52	<i>SIGSTU30</i>	Solyc07g056450.2	Solyc07g056450.3.1	1712	969	663	25.76	2	6.63	220	Cy ^{a,b}
53	<i>SIGSTU31</i>	Solyc07g056460.2	Solyc07g056460.3.1	3415	2719	663	25.51	2	5.76	220	Cy ^{a,b}
54	<i>SIGSTU32</i>	Solyc07g056470.2	Solyc07g056470.3.1	1024	951	663	25.58	2	5.57	220	Cy ^{a,b}
55	<i>SIGSTU33</i>	Solyc07g056480.2	Solyc07g056480.4.1	1654	1054	660	25.38	2	5.42	219	Cy ^{a,b}
56	<i>SIGSTU34</i>	Solyc07g056490.2	Solyc07g056490.4.1	1478	567	439	25.54	3	7.61	220	Cy ^{a,b}
57	<i>SIGSTU35</i>	Solyc07g056500.2	Solyc07g056500.3.1	1492	1030	660	25.41	2	6.10	219	Cy ^{a,b}

58	<i>SIGSTU36</i>	Solyc07g056510.2	Solyc07g056510.3.1	1836	715	672	26.04	2	4.95	223	Cy ^{a,b}
59	<i>SIGSTU37</i>	Solyc09g091140.2	Solyc09g091140.4.1	1359	666	666	25.28	2	5.31	221	Cy ^{a,b}
60	<i>SIGSTU38</i>	Solyc10g084960.2	Solyc10g084960.2.1	417	417	417	16.37	1	6.29	138	Pm ^a ,Cy ^b
61	<i>SIGSTU39</i>	Solyc08g062570.1	Solyc08g062570.1.1	1242	660	660	25.97	2	6.76	219	Cy ^{a,b}
62	<i>SIGSTU40</i>	Solyc10g007620.2	Solyc10g007620.3.1	1604	1031	666	25.84	2	5.79	221	Cy ^{a,b}
63	<i>SIGSTU41</i>	Solyc01g081310.2	Solyc01g081310.3.1	1531	1432	678	25.73	2	5.71	225	Cy ^{a,b}
64	<i>SIGSTU42</i>	Solyc06g069040.2	Solyc06g069040.4.1	4891	4499	681	25.96	2	5.15	226	Cy ^a ,Cp ^b
65	<i>SIGSTU43</i>	Solyc03g116130.1	Solyc03g116130.2.1	879	702	702	25.65	2	5.39	223	Cy ^{a,b}
66	<i>SIGSTU44</i>	Solyc03g116120.1	Solyc03g116120.3.1	740	675	675	25.88	2	5.93	224	Cy ^{a,b}
67	<i>SIGSTU45</i>	Solyc02g081240.1	Solyc02g081240.1.1	947	669	669	25.52	2	5.18	222	Cy ^a ,Nu ^b
68	<i>SIGSTU46</i>	Solyc01g099590.2	Solyc01g099590.4.1	1257	943	675	25.48	2	5.72	224	Cy ^{a,b} ,Cp ^a
69	<i>SIGSTU47</i>	Solyc01g086680.2	Solyc01g086680.4.1	1858	949	675	25.72	2	6.36	224	Cy ^{a,b}

Notes: Cp, Chloroplast. Cy, Cytoplasm. Mt, Mitochondria. Nu, Nucleus. Pm, Plasma-membrane.

Figures

Figure 1

RNA-seq data qualification. (a) Pairwise comparison of whole gene expression levels of four sets of samples with three biological replications. (b) Boxplot of overall expression levels of four sets of samples with three biological replications. The y-axis displays the log10 (FPKM) of every sample. (c) Venn diagram showing the number of DEGs in CK vs. ALA, LT vs. LTA, ALA vs. LTA, CK vs. LTA and CK vs. LT.

Figure 2

RNA-seq analysis of the four sets of samples. (a) GO annotation of DEGs. GO annotation of DEGs in CK vs. ALA, CK vs. LT, LT vs. LTA and CK vs. LTA. (b) KEGG pathways enrichment of DEGs. KEGG enrichment of DEGs in LT vs. LTA. (c) Expression analysis of SIGST genes in CK vs. ALA, CK vs. LT, LT vs. LTA and CK vs. LTA.

Figure 3

Chromosome mapping and tandem repeat analysis of SIGST gene. (a) Genomic distribution of SIGST genes on tomato chromosomes. The chromosome numbers and sizes (Mb) are indicated at the top and bottom of each bar, respectively. (b) A Circos plot illustrating the physical location of SIGST genes among the tomato chromosomes. Gray ribbons represent the putative duplicated segments from the whole genome duplication. Paralog pairs of SIGST genes are linked with red lines.

Figure 4

Analysis of protein domain and promoter. The SIGST genes circled in black box were differentially expressed in transcriptome.

Figure 5

Phylogenetic tree of GST genes from tomato, arabidopsis and rice. The red triangle represents the GST genes in tomato. The blue circle represents the GST genes in rice. Green diamond represents GST genes in Arabidopsis thaliana.

Figure 6

Expression of SIGSTs in tomato leaf under low temperature stress mediated by exogenous ALA.

Figure 7

Determination of GST activity and analysis of ROS content. (a) Determination of physiological indicators is GST activity, H₂O₂ content and O₂⁻ content. The experiments were repeated for three times. Different letters above the bars indicate a significant difference determined by one-way ANOVA with Tukey's test ($P < 0.05$). (b) Histochemical staining of H₂O₂ in tomato leaves at 24 h of treatment. The black spots represent the degree of accumulation of H₂O₂. (c) Histochemical staining of O₂⁻ in tomato leaves at 24 h of treatment. The black spots represent the degree of accumulation of O₂⁻.

Figure 8

Exogenous application of ALA under low temperature stress increases the tomato low temperature tolerance model by inducing SIGST genes expression and GST activity. The red plus sign indicates positive regulation.

Supplementary Files

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