

Table S1 RNA Qubit concentration and average size for each group, where C is the control and H is the heat-stressed leaves.

Sample Id	Qubit Conc	Avg size	Total nm
C1	14.13	544	39.35
C2	16.1	484	50.4
C3	15.2	514	44.87
H1	18.8	491	58.01
H2	13.61	553	37.29
H3	16.20	522	47.65

Table S2 Library preparations and sequencing description for control (C) and heat treated samples (H).

Sample Id	No. of Reads	Read length	GC%	Total data in GB
C11	50630508	150	54	15.1891524
C12	53953546	150	53	16.1860638
C21	50953446	150	52	16.1780629
C22	52953530	150	53	15.7691533
H11	52594531	150	54	15.7783593
H12	50502984	150	52	15.1508952
H21	51702981	150	53	15.1568944
H22	50703981	150	52	15.6568144

Table S3 Statistical analysis of differentially expressed genes (DEGs) in pearl millet leaves exposed to heat stress.

Mean	7.572638502
Standard Error	0.022718911
Median	8.184743854
Standard Deviation	3.350866095
Sample Variance	11.22830358
Kurtosis	-1.161610315
Skewness	-0.246996114
Range	16.57900415
Minimum	2
Maximum	18.57900415
Count	11483

Table S4 Pearl millet gene fold change in differentially expressed genes (DEGs) in heat-treated leaves compared with control samples matched by Arabidopsis orthologs.

Pearl millet accession	Fold change	Arabidopsis orthologs	Arabidopsis ortholog gene description
Pgl_GLEAN_10027569	16.3379328	AT2G34420	Photosystem II type I chlorophyll a/b-binding protein
Pgl_GLEAN_10034711	16.1868627	AT3G47470	Encodes a chlorophyll a/b-binding protein that is more similar to PSI Cab proteins than PSII
Pgl_GLEAN_10032833	15.0309001	AT3G14420	Aldolase-type TIM barrel family protein
Pgl_GLEAN_10032990	14.6687875	AT5G50920	Encodes a protein similar to the ATP-dependent Clp protease ATP binding subunit ClpC
Pgl_GLEAN_10032763	13.8572858	AT1G55490	Encodes the beta subunit of chloroplast chaperonin 60, a homolog of bacterial GroEL
Pgl_GLEAN_10018509	13.8434001	AT2G18960	Encodes plasma membrane proton ATPase
Pgl_GLEAN_10021526	13.7197486	AT4G24190	Encodes an ortholog of GRP94, an ER-resident HSP90-like protein, and is involved in the regulation of meristem size and organization
Pgl_GLEAN_10002651	13.6545662	AT5G49910	Stromal heat shock protein involved in protein import into the chloroplast
Pgl_GLEAN_10022408	13.5726233	AT5G56000	HEAT SHOCK PROTEIN 81.4 (Hsp81.4); FUNCTIONS IN: unfolded protein binding, ATP binding
Pgl_GLEAN_10029347	13.2505993	AT3G54050	Encodes a chloroplastic fructose 1,6-bisphosphate phosphatase.
Pgl_GLEAN_10037166	13.2377618	AT2G36530	Involved in light-dependent cold tolerance and encodes an enolase. Protein is tyrosine-phosphorylated, and its phosphorylation state is modulated in response to ABA in <i>A. thaliana</i> seeds
Pgl_GLEAN_10034557	13.2350161	AT5G01410	Encodes a protein predicted to function in tandem with PDX2 to form a glutamine amidotransferase complex involved in vitamin B6 biosynthesis.
Pgl_GLEAN_10036281	13.1855217	AT3G14940	Encodes a cytosolic phosphoenolpyruvate carboxylase (PEPC) that is active when expressed in <i>Escherichia coli</i> .

Pgl_GLEAN_10013653	13.1331319	AT1G68830	The predicted protein is approximately 20 amino acids shorter than most known Cab proteins. STN7 protein kinase, required for state transitions and phosphorylation of the major antenna complex
Pgl_GLEAN_10013122	13.0068145	AT5G56010	A member of the heat shock protein 90 (HSP90) gene family mediates the response and is crucial for protein import into the chloroplast stroma
Pgl_GLEAN_10006836	12.9150455	AT2G28000	Encodes chaperonin 60 alpha
Pgl_GLEAN_10006755	12.8604936	AT1G77490	Encodes the chloroplastic thylakoid ascorbate peroxidase tAPX
Pgl_GLEAN_10010682	12.8206846	AT2G04030	Encodes a chloroplast-targeted 90-kDa heat shock protein located in the stroma involved in red light. Encodes a chloroplast-targeted 90-kDa heat shock protein
Pgl_GLEAN_10029289	12.5749946	AT3G15360	Encodes prokaryotic thioredoxin
Pgl_GLEAN_10015409	12.4986565	AT5G03795	Exostosin family protein
Pgl_GLEAN_10014726	12.483517	AT5G62740	Hypersensitive-induced response protein 1 (HIR1)
Pgl_GLEAN_10033321	12.3766249	AT1G17290	Encodes alanine aminotransferase (ALAAT1), which is involved in alanine catabolism during plant recovery from hypoxia
Pgl_GLEAN_10018470	12.3564209	AT5G52640	Encodes the cytosolic heat shock protein AtHSP90.1. AtHSP90.1 interacts with the disease resistance signaling components SGT1b and RAR1 and is required for RPS2-mediated resistance.
Pgl_GLEAN_10012084	12.239033	AT2G24820	Translocon at the inner envelope membrane of chloroplasts 55-II (TIC55-II)
Pgl_GLEAN_10024877	12.2321299	AT5G15450	Encodes a chloroplast-targeted Hsp101 homolog
Pgl_GLEAN_10001789	12.1020842	AT2G21870	Encodes the FAd subunit of mitochondrial F1F0-ATP synthase.
Pgl_GLEAN_10028323	12.064962	AT3G04870	Required for anchoring the FNR flavoenzyme to the thylakoid membranes and sustaining high-efficiency photosynthetic linear electron flow.

			Involved in the biosynthesis of carotenes and xanthophylls, reduces zeta-carotene to lycopene.
Pgl_GLEAN_10031278	12.0624769	AT3G19450	Encodes a catalytically active cinnamyl alcohol dehydrogenase that uses p-coumaryl aldehyde as the preferred substrate.
Pgl_GLEAN_10033988	12.0583768	AT3G13580	Ribosomal protein L30/L7 family
Pgl_GLEAN_10023966	12.0348724	AT5G11720	Glycosyl hydrolase family 31 protein
Pgl_GLEAN_10033371	11.9887945	AT5G09590	Heat shock protein 70 (Hsc70-5)
Pgl_GLEAN_10007489	11.9703711	AT3G07770	Heat shock protein 89.1 (Hsp89.1)
Pgl_GLEAN_10015508	11.8934787	AT5G16150	Encodes a putative plastidic glucose transporter.
Pgl_GLEAN_10006025	11.8219905	AT4G16660	Heat shock protein 70 (Hsp 70) family protein
Pgl_GLEAN_10014986	11.8117319	AT4G31180	Class II aminoacyl-tRNA and biotin synthetase superfamily protein
Pgl_GLEAN_10016697	11.7778916	AT2G25140	Encodes ClpB4, which belongs to the casein lytic proteinase/heat shock protein 100 family (Clp/Hsp100)
Pgl_GLEAN_10021430	11.7518233	AT2G35490	Plastid-lipid associated protein PAP/fibrillin family protein
Pgl_GLEAN_10025601	11.6390618	AT5G35170	Adenylate kinase family protein
Pgl_GLEAN_10007875	11.6340885	AT3G06350	Encodes a bifunctional dehydroquinate-shikimate dehydrogenase enzyme that catalyzes two steps in the chorismate biosynthesis pathway.
Pgl_GLEAN_10027847	11.5848885	AT1G54320	Ligand-effect modulator 3 (LEM3) family protein/CDC50 family protein
Pgl_GLEAN_10005205	11.482938	AT5G20720	Encodes a chloroplast co-chaperonin similar to CPN21 from spinach and E. coli GroES.
Pgl_GLEAN_10007218	11.4812896	AT4G03200	Catalytics
Pgl_GLEAN_10026969	11.4089122	AT4G09040	RNA-binding (RRM/RBD/RNP motifs) family proteins
Pgl_GLEAN_10022988	11.3997667	AT5G42790	Encodes a protein with extensive homology to the largest subunit of the multicatalytic proteinase complex (proteasome).
Pgl_GLEAN_10017437	11.399642	AT3G10920	Manganese superoxide dismutase (MSD1)
Pgl_GLEAN_10001595	11.3818941	AT1G36280	L-Aspartase-like family protein

Pgl_GLEAN_10008425	11.3302679	AT1G53850	Encodes the alpha5 subunit of 20s proteasome involved in protein and RNA degradation.
Pgl_GLEAN_10030129	11.2308637	AT3G16910	Encodes a peroxisomal protein with acetyl-CoA synthetase activity that is responsible for the activation of acetate for entry into the glyoxylate cycle.
Pgl_GLEAN_10037787	11.1825782	AT5G52100	It is essential for chloroplast NAD(P)H dehydrogenase activity, which is involved in electron transfer between PSII and PSI.
Pgl_GLEAN_10007878	11.1357325	AT3G58140	Phenylalanyl-tRNA synthetase class IIc family protein
Pgl_GLEAN_10000489	11.0383137	AT1G07750	RmlC-like superfamily cupin protein
Pgl_GLEAN_10016081	11.0367949	AT5G40810	Cytochrome C1 family
Pgl_GLEAN_10031829	11.0009905	AT4G35450	Involved in the targeting of chloroplast outer membrane proteins to the chloroplast.
Pgl_GLEAN_10015411	10.7110047	AT2G14170	Arabidopsis thaliana methylmalonate-semialdehyde dehydrogenase
Pgl_GLEAN_10023896	10.6585562	AT1G17160	pfkB-like carbohydrate kinase family protein
Pgl_GLEAN_10017414	10.5840496	AT5G53400	Encodes BOBBER1 (BOB1), a non-canonical small heat shock protein required for development and thermotolerance
Pgl_GLEAN_10022182	10.5103678	AT2G15480	UDP-glucosyl transferase 73B5 (UGT73B5)
Pgl_GLEAN_10021406	10.4787487	AT2G35500	Encodes a protein with sequence similarity to shikimate kinases
Pgl_GLEAN_10032396	10.0593699	AT1G55020	Lipoxygenase
Pgl_GLEAN_10016566	9.98045073	AT1G23740	AOR is an alkenal/one oxidoreductase that acts on compounds with unsaturated alpha and beta carbonyls.
Pgl_GLEAN_10005520	9.94145571	AT5G02500	Encodes a member of the heat shock protein 70 family
Pgl_GLEAN_10024605	9.72883764	AT3G13860	Heat shock protein 60-3 A (HSP60-3A)
Pgl_GLEAN_10033184	9.5227851	AT3G10740	Encodes a bifunctional alpha-l-arabinofuranosidase/beta-d-xylosidase that belongs to the glycoside hydrolase family 51. It may be involved in cell wall modification.

Pgl_GLEAN_10014734	9.23896003	AT3G24420	Alpha/beta-hydrolase superfamily protein
Pgl_GLEAN_10007586	5.38789494	AT2G38810	Encodes the histone H2A protein HTA8
Pgl_GLEAN_10022809	3.44421884	AT4G10540	Subtilase family protein

Table S5 Description and classification of upregulated and downregulated gene protein functions and biological roles.

Description and classification of upregulated gene-related protein functions and their biological roles		
Protein class	Hits	%
Translational protein (PC00263)	1	12.50%
Metabolite interconversion enzyme (PC00262)	4	50.00%
No PANTHER category is assigned (UNCLASSIFIED)	2	25.00%
RNA metabolism protein (PC00031)	1	12.50%
Biological process		
Cellular process (GO:0009987)	4	50.00%
Metabolic process (GO:0008152)	4	50.00%
No PANTHER category is assigned (UNCLASSIFIED)	4	50.00%
Molecular function		
Binding (GO:0005488)	1	12.50%
Catalytic activity (GO:0003824)	5	62.50%
No PANTHER category is assigned (UNCLASSIFIED)	3	37.50%
Cellular component		
Cellular anatomical entity (GO:0110165)	2	25.00%
Protein-containing complex (GO:0032991)	1	12.50%
No PANTHER category is assigned (UNCLASSIFIED)	6	75.00%
Description and classification of downregulated genes related to protein function and their biological roles		
Protein class	Hits	%
Translational protein (PC00263)	1	12.50%
Metabolite interconversion enzyme (PC00262)	6	75.00%
No PANTHER category is assigned (UNCLASSIFIED)	1	12.50%
Biological process		
Response to stimulus (GO:0050896)	1	12.50%
Cellular process (GO:0009987)	4	50.00%
Metabolic process (GO:0008152)	4	50.00%
biological regulation (GO:0065007), TRANSLATION FACTOR GUF1-RELATED: translation initiation factor, ribosome binding, positive regulation of translation in the mitochondrion membrane (PTN000563148)	1	12.50%
No PANTHER category is assigned (UNCLASSIFIED)	3	37.50%
Molecular function		

Binding (GO:0005488)	3	37.50%
Catalytic activity (GO:0003824)	4	50.00%
No PANTHER category is assigned (UNCLASSIFIED)	1	12.50%

Cellular component

Cellular anatomical entity (GO:0110165)	2	25.00%
No PANTHER category is assigned (UNCLASSIFIED)	6	75.00%
