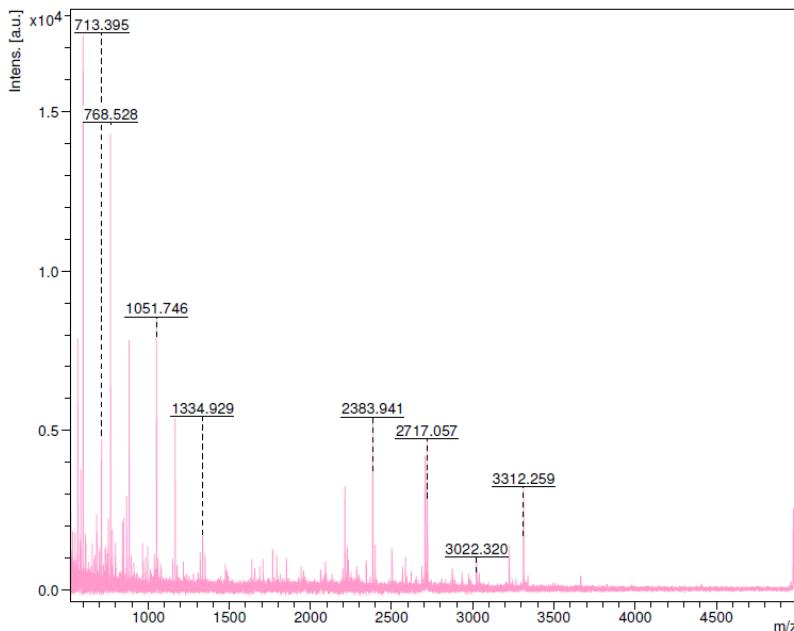


Supplementary Data

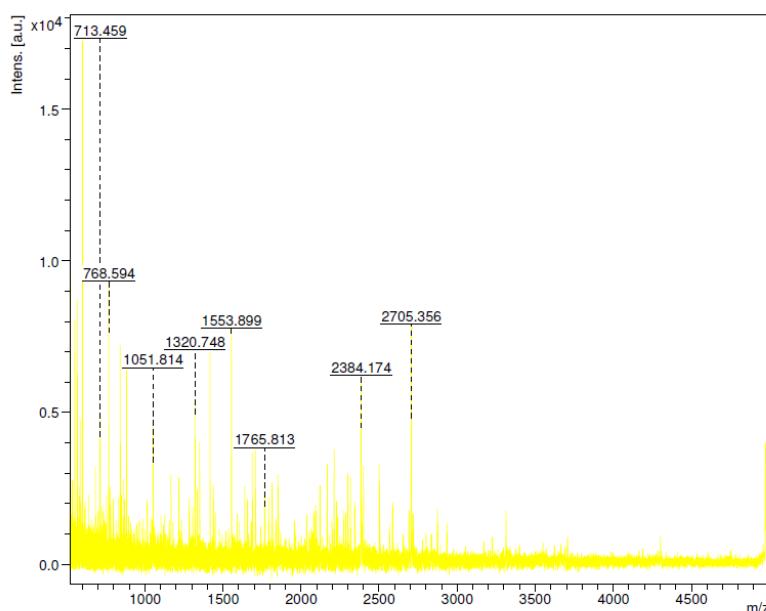
Proteome analysis of wheat (*Triticum aestivum*) for the identification of differentially expressed heat-responsive proteins

Ranjeet Ranjan Kumar*, Gyanendra Pratap Singh, Suneha Goswami, Himanshu Pathak and Raj Deo Rai



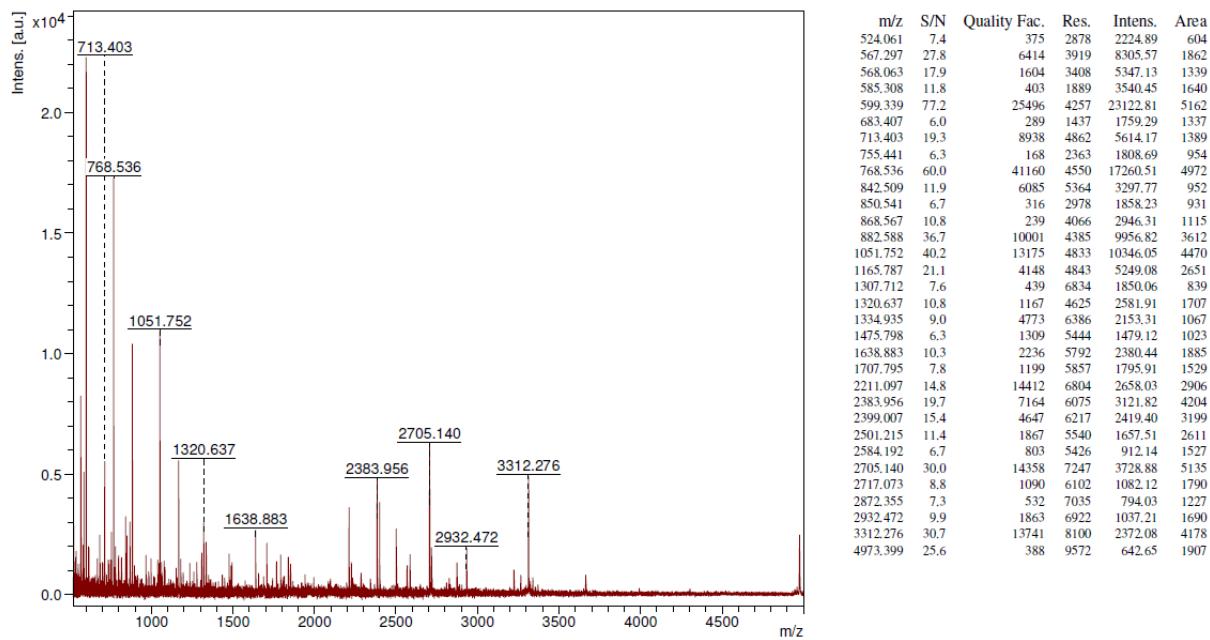
m/z	S/N	Quality	Fac.	Res.	Intens.	Area
524.063	8.4		212	2427	2294.74	735
567.289	28.0		2937	3532	7508.94	1838
568.049	20.3		921	3217	5433.77	1436
585.291	8.6		145	1254	2283.51	1590
586.330	6.7		146	2773	1778.96	562
599.331	65.1		43380	3651	17266.41	4386
683.399	6.0		539	1226	1538.83	1361
685.342	6.1		739	4891	1552.30	362
713.395	19.1		4859	4608	4820.07	1261
755.431	6.2		153	2196	1543.66	877
768.528	58.5		20800	4269	14576.37	4510
780.429	7.1		466	2538	1742.74	932
842.496	7.9		2478	4446	1872.91	621
850.526	6.9		349	2645	1613.36	908
868.553	12.5		350	4048	2902.26	1112
882.581	32.3		8383	4232	7422.38	2771
996.638	6.1		1156	5622	1312.82	450
1051.746	38.1		9414	4473	7911.95	3677
1165.792	26.9		45747	5611	5353.00	2386
1334.929	8.9		863	5556	1700.11	943
2211.095	16.0		3775	6032	2433.95	2979
2225.104	6.1		1251	5981	920.82	1141
2383.941	28.0		15270	6664	3710.69	4624
2398.998	7.6		2998	6691	1001.31	1251
2501.208	6.9		2238	4907	830.40	1486
2705.132	25.0		7088	6807	2607.77	3862
2717.057	27.7		4263	6730	2848.47	4296
3022.320	6.8		2445	6752	538.05	958
3223.236	10.0		2577	7493	662.59	1205
3312.259	26.7		7708	8895	1670.72	2772
4973.387	30.4		1223	9200	625.77	1893

Supplementary Figure 1(a). Peptide spectrum of protein spot (ID 14) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.

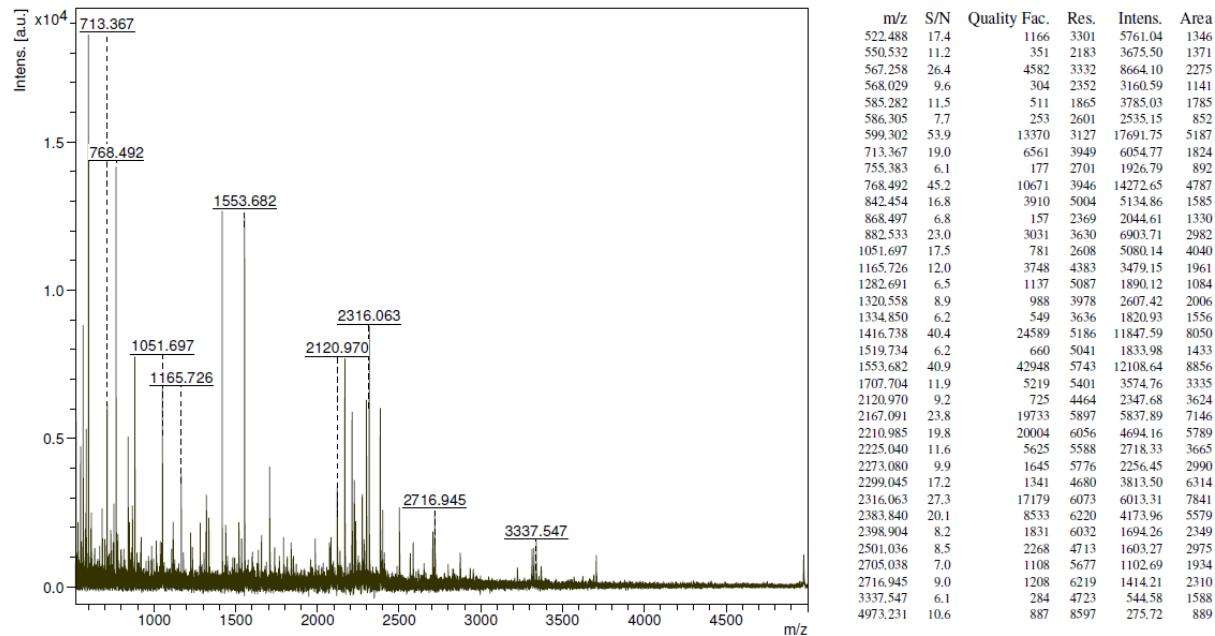


m/z	S/N	Quality	Fac.	Res.	Intens.	Area
522.491	12.9		734	1416	6461.99	3442
523.097	6.1		131	1747	3070.48	1336
524.154	6.1		116	2310	3060.68	1045
550.541	10.7		359	1364	5303.69	3149
567.301	10.5		522	1041	5165.93	4158
568.179	10.4		446	1995	5168.31	2259
599.389	30.7		10578	1682	15059.99	8305
713.459	8.8		4700	2042	4188.83	2389
768.594	16.1		2683	1640	7610.27	6001
842.554	14.0		4751	2566	6394.04	3713
882.624	10.4		1153	1564	4696.39	4722
1051.814	7.6		292	1317	3346.05	5180
1320.748	11.2		6219	2987	4934.44	5135
1350.853	7.6		5789	3090	3368.29	3540
1416.937	14.1		13625	3248	6289.92	6800
1553.899	16.9		62785	3817	7591.15	8442
1688.129	6.5		1341	2816	2953.23	5090
1707.957	7.1		1806	3137	3210.83	5129
1765.813	6.3		590	3281	1897.81	4726
2211.304	7.7		10010	4336	2709.66	4824
2384.174	14.3		6464	4095	4475.63	9254
2399.209	6.6		1507	3564	2117.05	4759
2501.432	7.2		3163	3411	2068.41	5221
2705.356	19.9		32978	4205	4791.29	11538
4973.360	15.6		1590	3970	669.87	4971

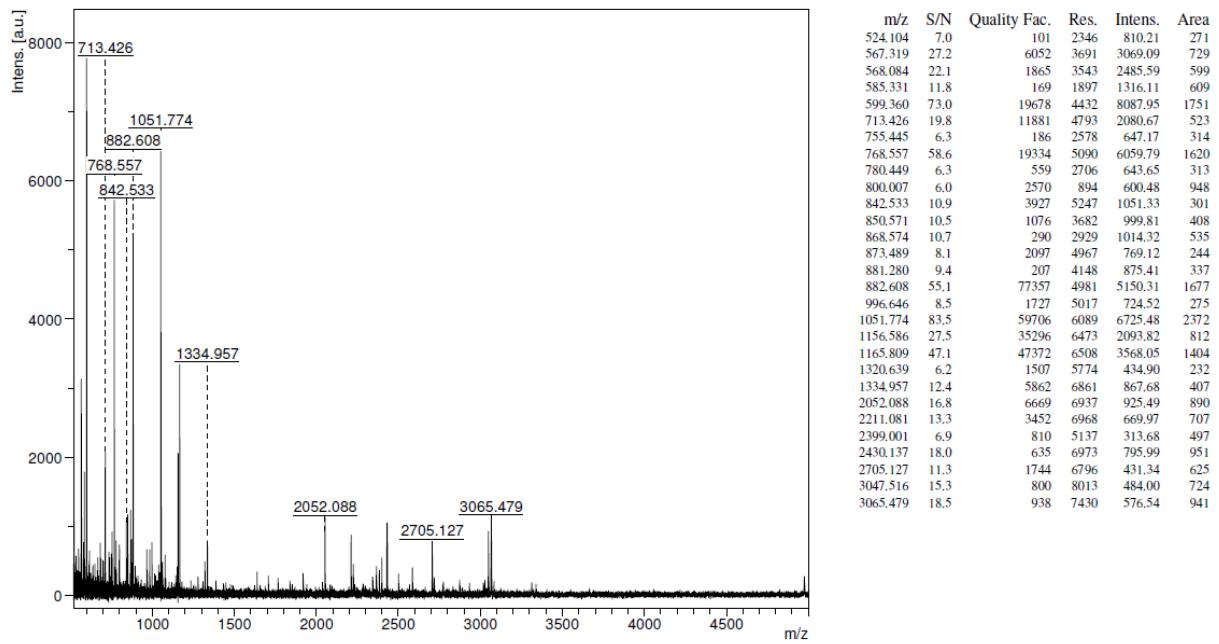
Supplementary Figure 1(b). Peptide spectrum of protein spot (ID 15) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



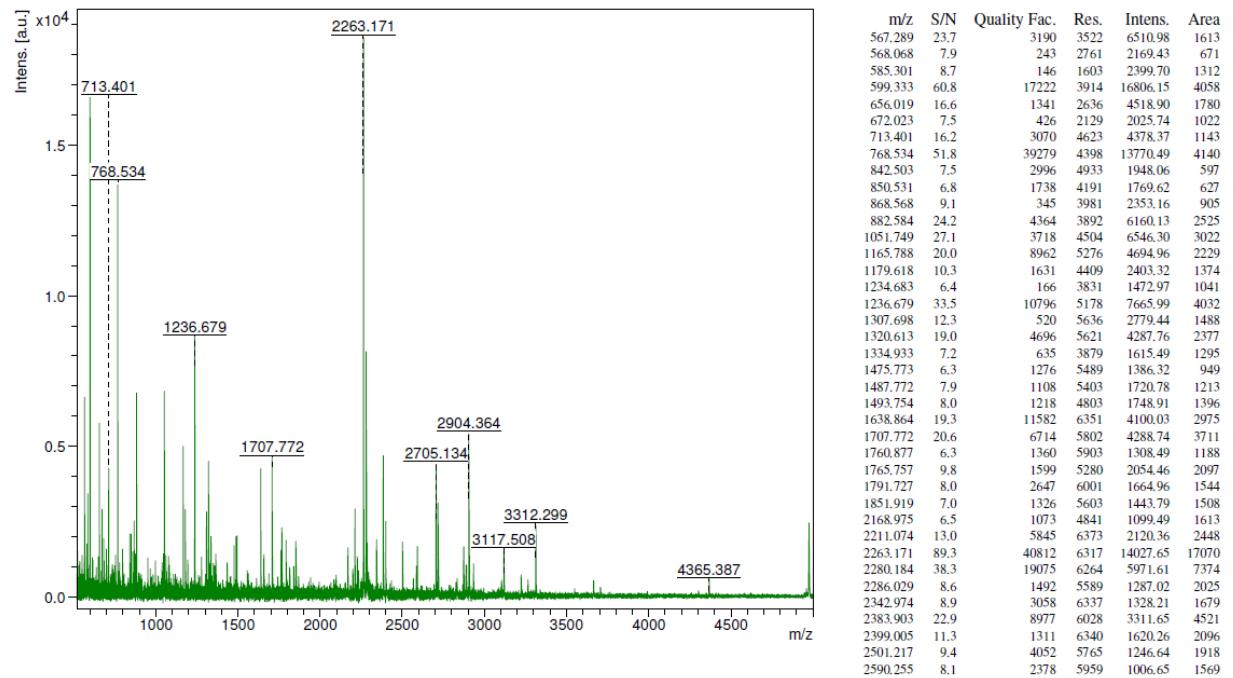
Supplementary Figure 1(c). Peptide spectrum of protein spot (ID 16) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



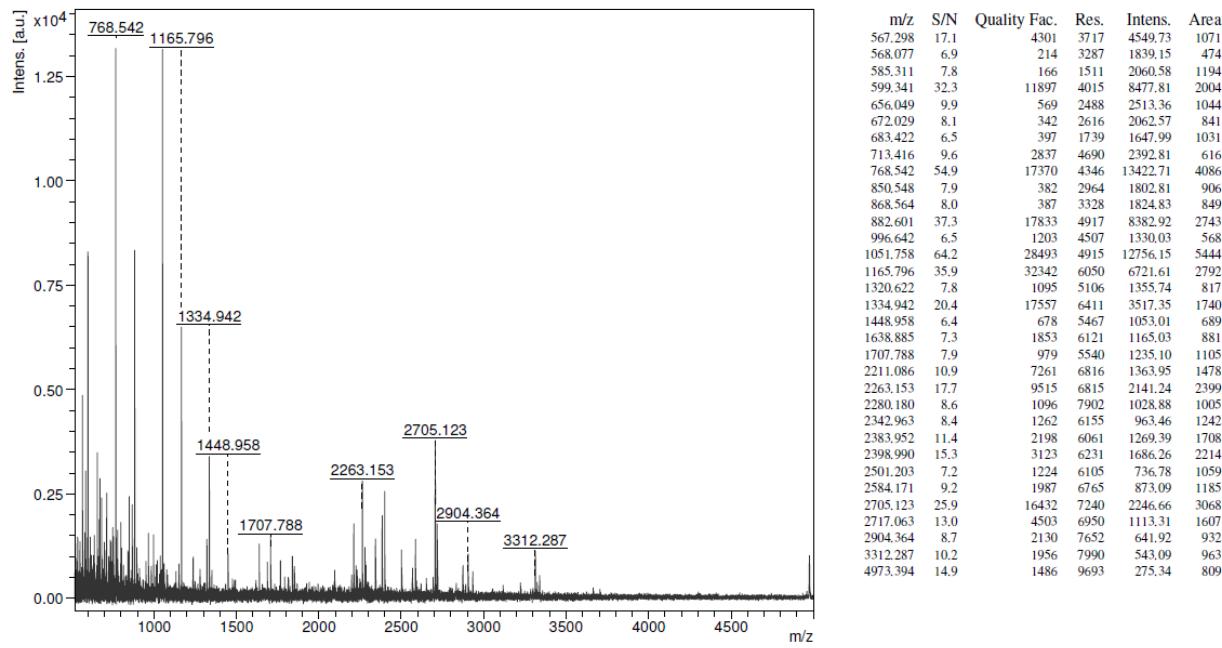
Supplementary Figure 1(d). Peptide spectrum of protein spot (ID 20) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



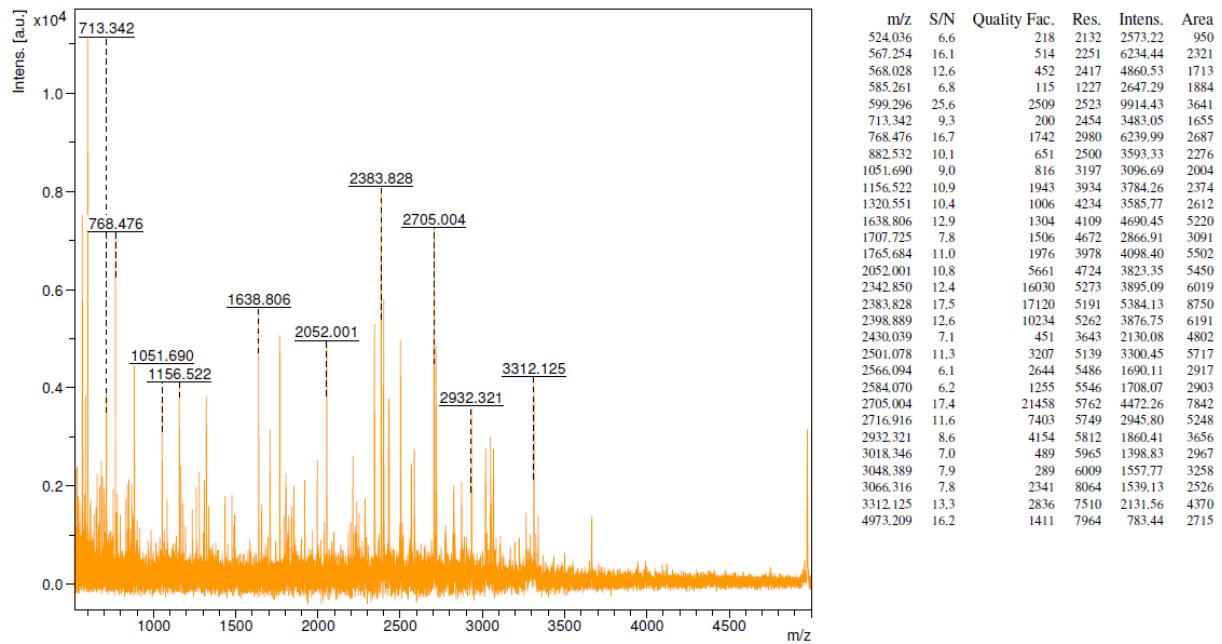
Supplementary Figure 1(e). Peptide spectrum of protein spot (ID 32) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



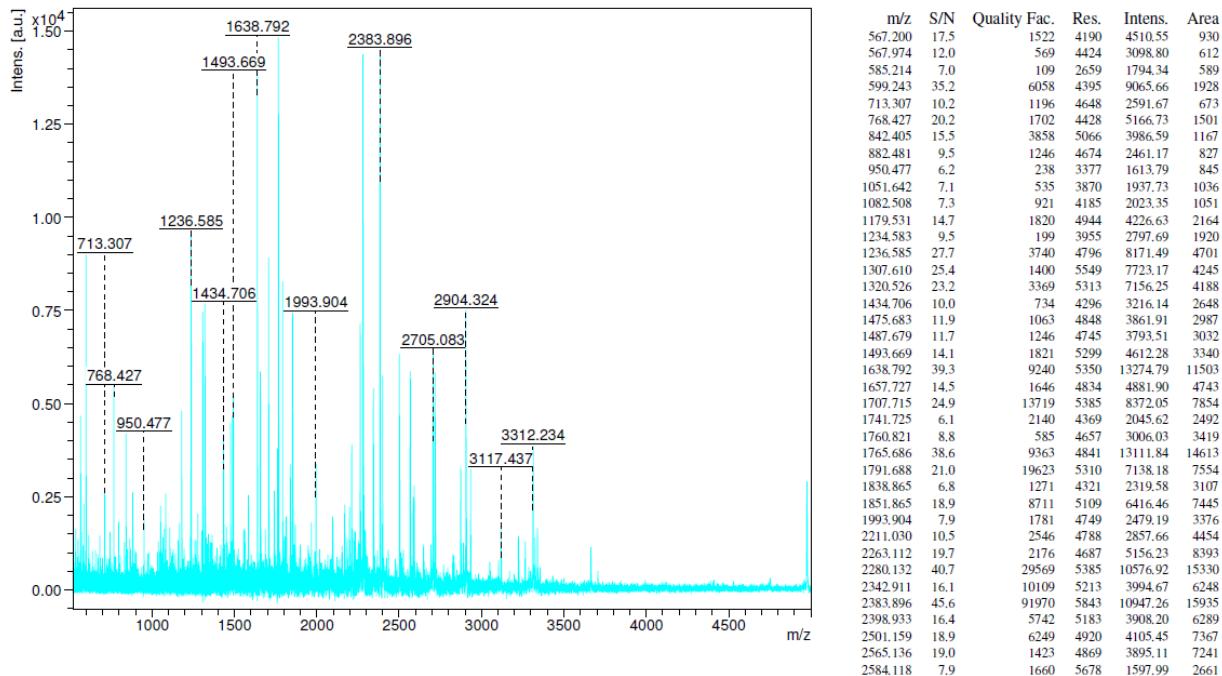
Supplementary Figure 1(f). Peptide spectrum of protein spot (ID 35) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



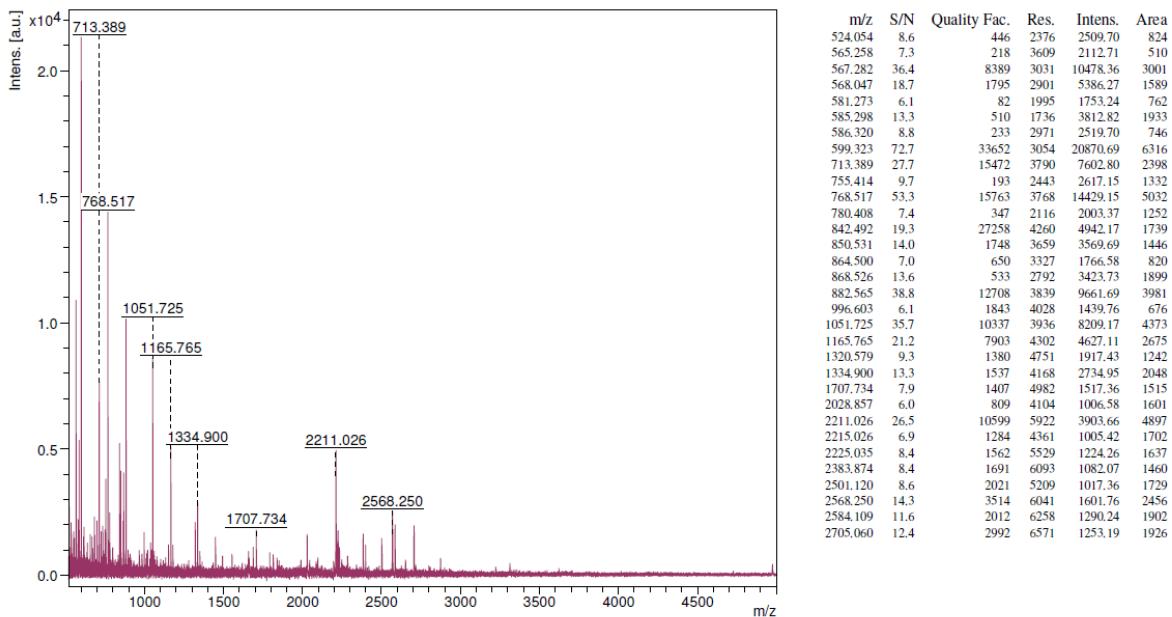
Supplementary Figure 1(g). Peptide spectrum of protein spot (ID 36) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



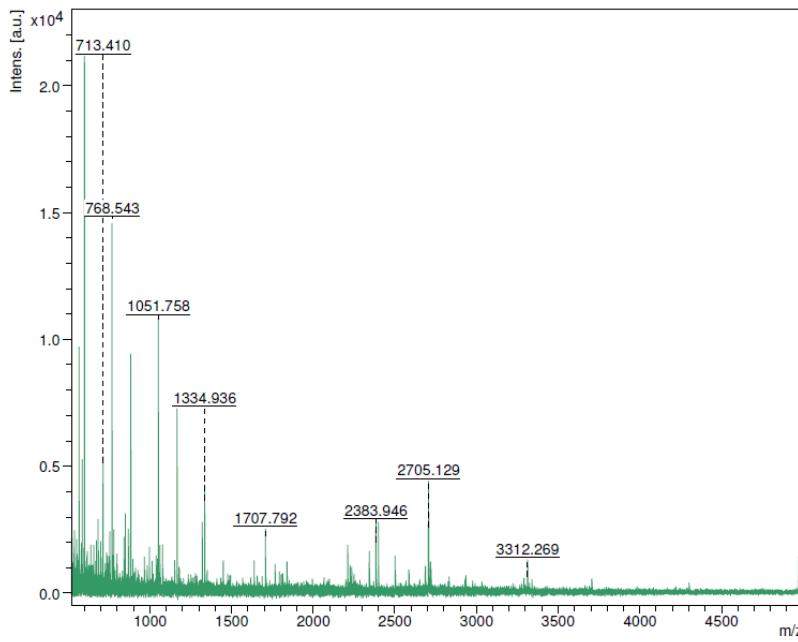
Supplementary Figure 1(h). Peptide spectrum of protein spot (ID 45) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



Supplementary Figure 1(i). Peptide spectrum of protein spot (ID 52) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.

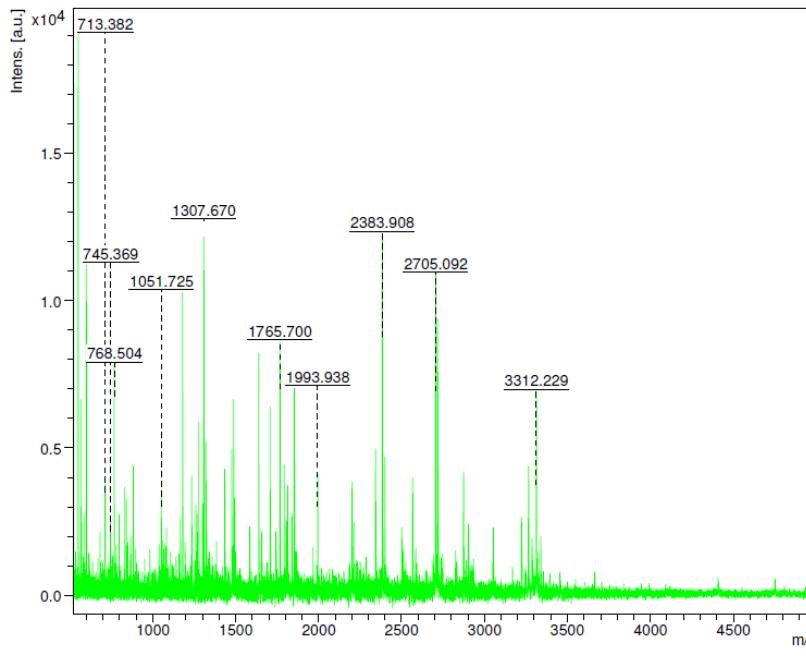


Supplementary Figure 1(j). Peptide spectrum of protein spot (ID 66) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



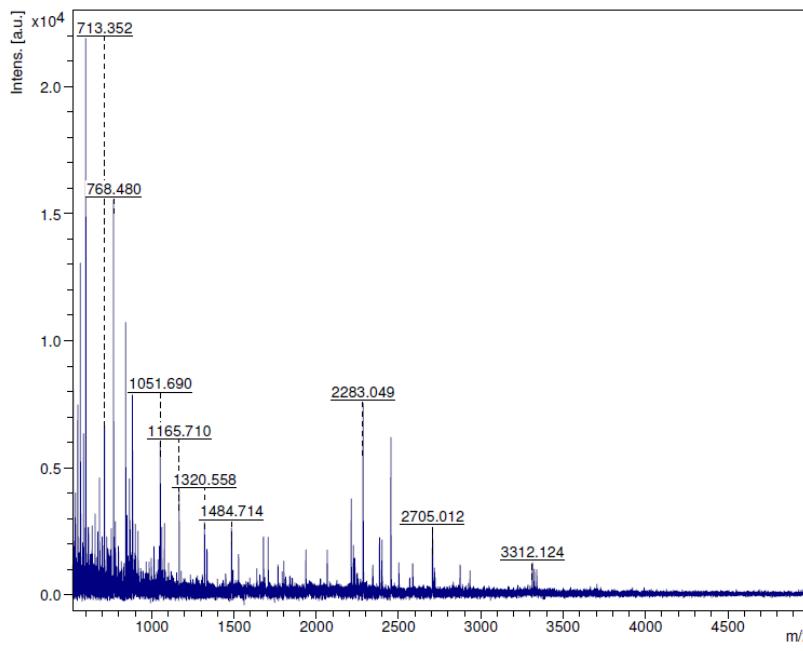
m/z	S/N	Quality Fac.	Res.	Intens.	Area
523.089	6.4	153	1920	2400.09	992
567.300	26.1	5646	3777	9632.38	2244
585.311	10.9	188	1985	3969.62	1749
599.343	60.5	10517	3998	21953.90	5244
683.408	6.8	294	2288	2393.28	1136
713.410	14.6	8364	4496	5109.57	1371
768.543	42.7	26154	4386	14742.21	4459
842.509	6.3	2078	5019	2088.88	630
850.542	7.1	384	2764	2322.63	1255
882.593	24.6	3258	3464	8007.60	3670
1051.758	36.5	14384	4755	10761.22	4700
1165.794	24.6	11675	4811	6891.34	3525
1320.635	9.2	1539	4473	2430.03	1671
1334.936	13.8	2904	4601	3639.77	2477
1707.792	9.1	1293	5434	2219.54	2034
2211.090	6.6	1393	5286	1263.60	1766
2383.946	11.7	4314	6395	1996.56	2598
2398.986	11.1	2565	6279	1886.68	2480
2705.129	18.8	2886	6539	2572.71	3949
3312.269	7.2	1129	8161	6294.41	1166
4973.419	13.0	1147	9212	422.89	1274

Supplementary Fig 1(k). Peptide spectrum of protein spot (ID 74) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



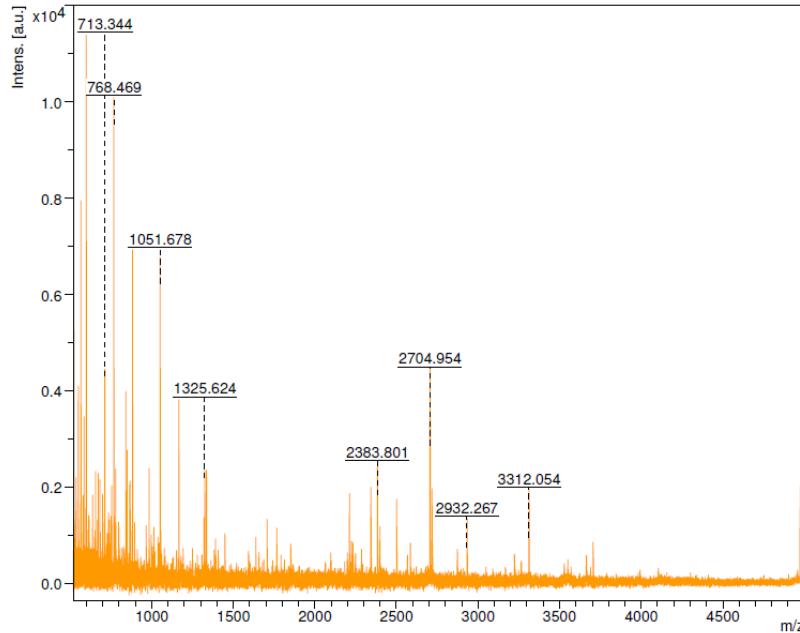
m/z	S/N	Quality Fac.	Res.	Intens.	Area
522.481	48.4	8849	2287	16310.69	5489
523.471	11.4	1319	3230	3851.24	900
524.054	6.4	374	2355	2136.40	738
550.527	52.2	6512	2302	17365.17	6203
551.507	11.3	289	2551	3761.44	1187
567.280	18.9	2460	3032	6292.08	1803
568.046	7.4	404	1794	2467.67	1165
599.312	34.3	3483	3327	11403.95	3257
713.382	10.5	2243	3433	3480.78	1171
745.369	6.5	2193	4070	2160.69	657
768.504	20.3	1413	3062	6741.27	2811
832.453	9.6	1625	3337	3173.96	1393
842.473	8.8	3053	3571	2930.49	1224
882.259	10.7	1089	3059	3559.52	1844
1051.725	8.6	846	3294	3010.03	1897
1165.710	6.4	1324	3311	2345.77	1770
1179.596	25.6	11400	4092	9374.39	5844
1234.627	7.6	218	2339	2861.44	3282
1277.698	14.0	2937	4098	5313.06	3761
1300.565	6.1	151	2709	2350.58	2556
1307.670	32.8	6163	4643	12672.50	8356
1320.575	11.7	606	3926	4535.41	3538
1434.756	8.5	1047	3955	3413.10	5098
1475.756	9.4	274	2956	3834.65	4845
1487.738	13.4	2478	4077	5472.30	5098
1493.719	6.5	573	3975	2658.06	2551
1638.835	16.3	2612	4049	6923.69	7830
1707.747	13.0	5323	4315	5462.85	6346
1765.700	16.5	4997	4268	6984.66	8728
1791.693	7.5	836	3736	3174.84	4628
1851.894	13.2	1981	4093	5655.23	8128
1993.938	7.5	1423	4259	2992.46	4528
2201.110	7.7	2514	4425	2718.01	4616
2342.941	10.7	7775	5365	3502.07	5396
2383.908	27.4	18452	5087	8744.56	14405
2398.969	9.0	1340	4348	2826.23	5356
2565.118	9.1	1299	4839	2551.95	4763
2705.092	27.0	26332	5517	6899.54	12682
2717.009	23.4	21055	5654	5911.46	10712

Supplementary Figure 1(l). Peptide spectrum of protein spot (ID 84) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



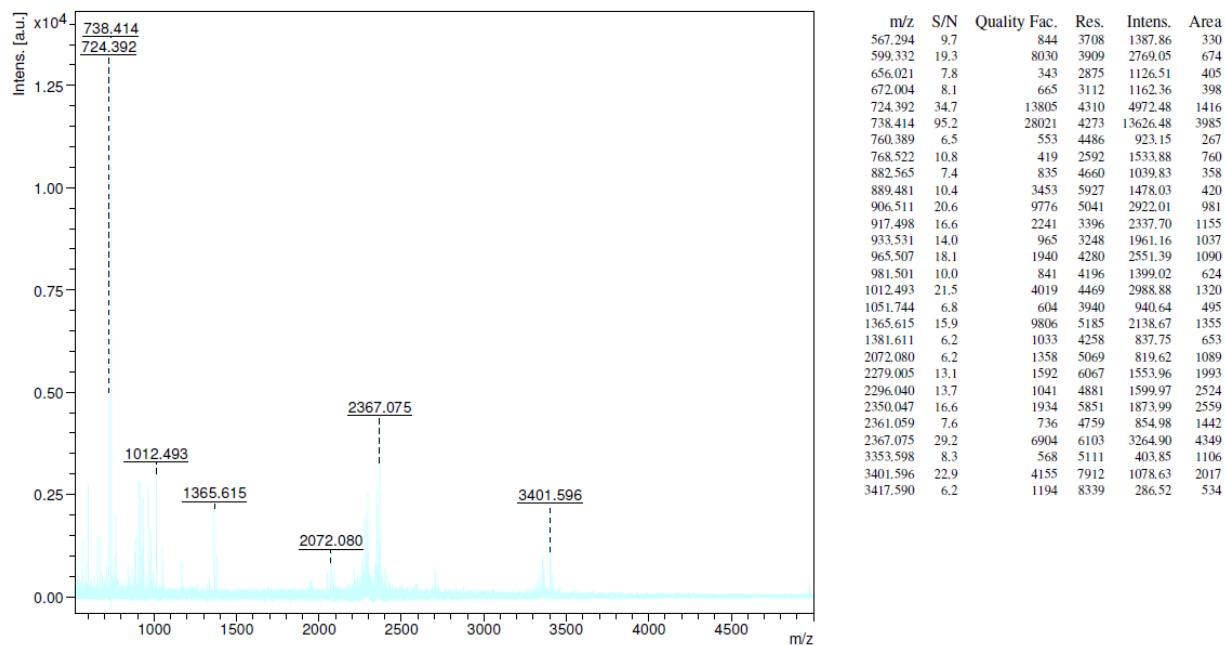
m/z	S/N	Quality	Fac.	Res.	Intens.	Area
522.463	15.9		3037	3198	7905.26	1875
537.173	6.8		82	2760	3352.99	958
550.500	12.8		587	2713	6239.19	1862
567.251	25.7		4002	3308	12537.36	3256
568.031	9.1		163	2786	4464.35	1359
585.268	9.6		157	1721	4705.93	2401
599.288	43.3		23764	3394	21268.41	5873
683.349	6.8		421	1761	3175.93	1966
713.352	14.2		7594	4362	6589.69	1836
768.480	32.9		9245	3879	14976.13	5020
842.451	24.2		52917	4654	10475.79	3419
864.439	7.6		463	3092	3216.22	1597
882.532	16.6		1603	3559	6957.04	3103
1051.690	14.6		2121	3876	5436.34	2922
1165.710	9.4		870	3838	3313.07	2122
1320.558	8.0		1659	4489	2556.69	1759
1484.714	6.2		774	1537	1879.20	4574
2210.989	10.9		3090	5770	2584.96	3299
2283.049	24.4		8616	5748	5454.07	7345
2383.842	6.7		982	4251	1372.87	2657
2398.890	6.0		1241	4852	1235.05	2118
2452.053	20.5		10498	6048	4060.22	5762
2705.012	10.0		1836	5604	1600.99	2850
3312.124	6.0		1136	7001	594.83	1274
4973.129	6.8		875	8671	225.54	733

Supplementary Figure 1(m). Peptide spectrum of protein spot (ID 118) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



m/z	S/N	Quality	Fac.	Res.	Intens.	Area
522.459	15.5		1754	2700	4869.60	1379
523.026	8.3		173	2037	2624.08	1001
550.501	10.8		341	2587	3345.45	1048
567.248	25.5		2213	3693	7864.19	1875
568.030	10.3		369	2397	3196.97	1135
585.262	7.7		154	1400	2380.28	1483
599.283	35.2		8205	3215	10807.69	3093
713.344	14.6		7636	4073	4294.31	1260
768.469	33.0		4704	3710	9519.67	3343
780.358	6.2		451	1794	1795.19	1305
842.432	12.9		4405	4183	3540.47	1258
850.481	7.8		618	3001	2117.31	1055
882.517	21.3		2738	3310	5729.76	2735
983.459	7.9		189	2636	2039.77	1443
1051.678	24.9		6235	4156	6215.20	3126
1165.704	14.7		4859	4416	3504.09	1975
1325.624	9.7		4853	5032	2175.55	1348
1334.834	9.6		769	4391	2156.13	1528
2210.943	6.6		956	4423	1090.98	1792
2342.821	8.9		1884	5788	1361.26	1882
2383.801	12.2		3663	5696	1826.01	2662
2501.045	7.6		2808	5559	1050.08	1678
2704.954	23.5		20397	7040	2857.78	4120
2716.875	9.3		1135	5929	1114.04	1864
2932.267	7.1		1528	6438	735.89	1304
3312.054	11.7		8110	8299	948.62	1681
4973.064	17.3		1807	8548	482.89	1587

Supplementary Figure 1(n). Peptide spectrum of protein spot (ID 120) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.



Supplementary Figure 1(p). Peptide spectrum of protein spot (ID 164) from 2-Dimensional Electrophoresis (2-DE) gel of HD2985 cultivar of wheat using MALDI-TOF/MS.

Supplementary Table 6. Sample info and running conditions followed for normal liquid chromatography mass spectrometry (nLC-MS/MS) for identification of peptides and proteins in control (22°C) and heat shock treated (42°C, 2 h) samples of HD2985 cultivar of wheat using available protein database, (a) *Arabidopsis thaliana* protein database (b) *Oryza sativa* protein database (NCBI RefSeq).

(a)	
Sample Name	HD2985 (Control) and HD2985(Treated)
MS run details and parameters	C18 nLC column used for separation of peptides
Run time (gradient time)	120 min
Resolution settings for MS and MS2	MS @60000 & MS2@15000
Top N	20
Fragmentation method	HCD
Search parameters	
Database	<i>Arabidopsis thaliana</i> protein database (NCBI RefSeq)
Search algorithm	Sequest
Modifications	methionine oxidation : dynamic & Carbamidomethyl at cysteine : static
Enzyme	Trypsin
Missed cleavages	1
MS and MS/MS tolerances	MS : 20 ppm and MS/MS : 0.1 Da

(b)	
Sample Info	HD2985 (Control) and HD2985 (Treated)
LC details	
Enrichment column	75um i.d.; 2 cm length; 5um C18 particles
Analysis column	75um i.d.; 10 cm length; 3um C18 particles
LC gradient	0 to 90min - 5 to 30% Solvent B
MS run details and parameters	

Run time (gradient time)	120 min
Resolution settings for MS and MS2	MS@60000 & MS2@15000
Top N	20
Fragmentation method	HCD
Search parameters	
Database	<i>Oryza sativa (rice)</i> protein database (<i>NCBI RefSeq</i>)
Search algorithm	Sequest
Modifications	methionine oxidation : dynamic & Carbamidomethyl at cysteine : static
Enzyme	Trypsin
Missed cleavages	1
MS and MS/MS tolerances	MS : 20 ppm and MS/MS : 0.1 Da

Supplementary Table 7. List of primers used for characterizing the expression pattern of identified heat stress associated proteins in control and heat shock treated samples of wheat (cv. HD2985) using quantitative real time PCR.

Primers	Sequences (5'-3')	Tm (°C)
HSP90f	TGAGTATGGGTGGACTGCCAACAT	62.7
HSP90r	TCTCGAAGAGCAGCATACAAGGT	62.7
HSP70f	CTTCGTCCAGGAGTTCAAGC	63.9
HSP70r	GTCGATCTCGATGGTGGTTT	63.9
HSP26f	ATCGACGTGAAGGTCCAGTGATGA	62.7
HSP26r	AGAAAGGATCGGAGAAGAACGGCA	62.7
CATf	CAAGAGCGATTCAACAGAT	63
CATr	AGACCAGTAGGAGAGCCAGATG	63.6
Can3714f	GGCCGGTCATTCATAGAGAA	60.04
Can3714r	TGAATTGGACCAAAAGGAG	60.04
SIPf	TCCCCAAGAACTTCAAGCAC	60.2
SIPr	CTTCTCCCCTTCCTGAAGC	60.3
RCAf	CGCAAGTACGACTTCGACAA	57.3
RCAr	CAGGATGAGAGGGACCTTGA	59.4
CDPKf	TGCCATTCACTGGTGTGAT	55.3
CDPKr	GGCCTGGCTTGAAGTAGATG	59.4
Calf	TTTCGAGCACCTCAAGGAGT	59.9
Calr	TCTTCAGCTGCATTGGTGTGTC	59.9
AGPf	ACAGCCTTCAAAGTTCAGCTTC	60
AGPr	GTGCTCTACCCCTGCATTATCC	59.9
GAPDHf	CGGAAAGTTGACTGGAATGG	64.3
GAPDHR	ATCATAGGTTGCTGGCTTCG	64
Can152f	GCAAGGCTGAACTAGACAGGTT	59.9

Can152r	CCTCTGCAATTCCCTCTTGAT	59.8
Actf	GCGGTCGAACAACTGGTATT	63.7
Actr	GGTCCAAACGAAGGATAGCA	63.8

*f-forward; r-reverse; CAT-catalase; SIP- stress induced protein; Can- candidate gene; RCA- rubisco activase; CDPK- calcium dependent protein kinase; AGP- ADP glucopyrophosphorylase; GAPDH- glyceraldehyde-3-phosphate dehydrogenase; Act- actin.