**Transcriptome Analysis of a *Triticum aestivum* Landrace (*Roshan)* in Response to Salt Stress Conditions**

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( DOCX)

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**Figure S1. The top 10 significant enriched pathways with their related number of genes.**

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**Figure S2. Validation of selected genes(TraesCS3A02G363700 (pyrroline-5-carboxylate synthetase), TraesCS2D02G493700 (serine-threonine protein kinase), TraesCS5B02G210100 ( Aldehyde dehydrogenase family 3 member I1, chloroplastic-like), TraesCS1D02G212400( Proline dehydrogenase 2, mitochondrial like), TraesCS7B02G335200 (Putative calcium binding protein CML29), TraesCS7D02G094900 ( GDSL\_esterase/lipase\_At2g40250-like)) using qRT-PCR**

**Table S1. List of primers for real-time PCR and their sequences.**

|  |
| --- |
| PRIMER PICKING RESULTS FOR TraesCS3A02G363700|TraesCS3A02G363700.3  Definition: pyrroline-5-carboxylate synthetase  Template masking not selected  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_start)  [len](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_len)  [tm](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_tm)  [gc%](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_gc)  [an](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_any)y  [3'](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_three) [hairpin](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_hairpin) [seq](http://primer3.ut.ee/cgi-bin/primer3/primer3/primer3web_help.cgi#p3w_primer_seq) Annealing temperature  LEFT PRIMER 2129 20 58.73 50.00 0.00 0.00 0.00 TCTGTGCGAGGAATGAAGGA 57  RIGHT PRIMER 2247 23 58.26 43.48 0.00 0.00 0.00 CAACTAGTGCCATAACTACGACA  SEQUENCE SIZE: 2342  INCLUDED REGION SIZE: 2342  PRODUCT SIZE: 119, PAIR ANY\_TH COMPL: 0.00, PAIR 3'\_TH COMPL: 0.00 |
| RIMER PICKING RESULTS FOR TraesCS2D02G493700|TraesCS2D02G493700.1  Definition: serine-threonine protein kinase  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ) Annealing temperature  LEFT PRIMER 1208 20 58.86 50.00 4.00 0.00 ACGTGTGGATGTGTTCCTGT 63  RIGHT PRIMER 1302 20 58.77 45.00 4.00 0.00 TTTACACAGCAAGGCCAATC  SEQUENCE SIZE: 1404  INCLUDED REGION SIZE: 1404  PRODUCT SIZE: 95, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 3.00 |
| PRIMER PICKING RESULTS FOR TraesCS5B02G210100|TraesCS5B02G210100.1  Definition: Aldehyde dehydrogenase family 3 member I1, chloroplastic-like  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ) Annealing temperature  LEFT PRIMER 2572 20 58.92 55.00 4.00 3.00 AGCCATCTGTGCCCTAGTCT 59  RIGHT PRIMER 2668 20 59.16 55.00 8.00 3.00 CGACCGGTCTGATCTAAGGT  SEQUENCE SIZE: 2803  INCLUDED REGION SIZE: 2803  PRODUCT SIZE: 97, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 2.00 |
| PRIMER PICKING RESULTS FOR TraesCS1D02G212400|TraesCS1D02G212400.1  Definition: Proline dehydrogenase 2, mitochondrial like  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ) Annealing temperature  LEFT PRIMER 1705 20 58.70 55.00 3.00 0.00 AGAAGCGACGGAGTTAGGAG 55  RIGHT PRIMER 1801 20 59.29 45.00 4.00 1.00 AGCCTGACGATTGAACAACA  SEQUENCE SIZE: 1974  INCLUDED REGION SIZE: 1974  PRODUCT SIZE: 97, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 0.00 |
| PRIMER PICKING RESULTS FOR TraesCS7B02G335200|TraesCS7B02G335200.1  Definition: Putative calcium binding protein CML29  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ) Annealing temperature  LEFT PRIMER 641 20 58.32 55.00 8.00 2.00 CTAGCTAGAGGGCGTGCATA 57  RIGHT PRIMER 720 20 59.86 55.00 4.00 2.00 TCCACAACTCTCCTGACGTG  SEQUENCE SIZE: 757  INCLUDED REGION SIZE: 757  PRODUCT SIZE: 80, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 0.00 |
| PRIMER PICKING RESULTS FOR TraesCS7D02G094900|TraesCS7D02G094900.1  Definition: GDSL\_esterase/lipase\_At2g40250-like  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ) Annealing temperature  LEFT PRIMER 1168 20 59.03 50.00 6.00 2.00 GCTGCAGATCGACCAACTTA 62  RIGHT PRIMER 1258 20 58.57 50.00 5.00 3.00 GGGCTCACACATTGTAGGAA  SEQUENCE SIZE: 1351  INCLUDED REGION SIZE: 1351  PRODUCT SIZE: 91, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00 |
| PRIMER PICKING RESULTS FOR TraesCS2A02G539700.1 TraesCS2A02G539700:TraesCS2A02G539700.1 cdna:protein\_coding  Definition: ACTIN2 (HOUSEKEEPING GENE)  No mispriming library specified  Using 1-based sequence positions  OLIGO [start](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_START)  [len](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_LEN)  [tm](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_TM)  [gc%](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_GC)  [any](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_ANY)  [3'](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_REPEAT) [seq](http://bioinfo.ut.ee/primer3-0.4.0/primer3_www_results_help.html#PRIMER_OLIGO_SEQ) Annealing temperature  LEFT PRIMER 1489 20 57.83 50.00 8.00 3.00 GCAGCTATAACTGGCAAAGC 60  RIGHT PRIMER 1579 20 58.14 50.00 3.00 2.00 ACACCTTGGACTGATGCTGT  SEQUENCE SIZE: 1714  INCLUDED REGION SIZE: 1714  PRODUCT SIZE: 91, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 3.00  EXCLUDED REGIONS (start, len)\*: 1,1422 1600,114 |

**Table S2. Summary of transcriptome sequencing and mapping by using STAR and HISAT2 for local salt tolerant wheat landrace (Roshan).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| STAR | | | | | | |
| **Sample name** | **Clean Reads** | **Uniquely**  **mapped** | **Mapped in**  **multiple loci** | **Mapped in**  **too many loci** | **Overall Alignment**  **Rate** |  |
| Sample\_I(Normal) | 23,060,971 | 18,418,485 | 26,233,47 | 13,189 | 91.30% |  |
| Sample\_II(Normal) | 22,932,551 | 18,925,319 | 20,529,59 | 13,533 | 91.54% |  |
| Sample\_III(Normal) | 23,202,034 | 19,276,439 | 18,472,27 | 18,885 | 91.12% |  |
| Sample\_I(Stress) | 22,853,565 | 18,423,202 | 12,756,35 | 20,854 | 86.29% |  |
| Sample\_II(Stress) | 22,789,255 | 18,862,977 | 12,198,27 | 20,903 | 88.22% |  |
| Sample\_III(Stress) | 22,670,166 | 18,831,868 | 13,542,43 | 19,979 | 89.13% |  |
| HISAT 2 | | | | | | |
| **Sample name** | **Clean Reads** | **Concordantly1** | **Concordantly >1** | **Discordantly1** | **Discordantly >1** | **Overall Alignment**  **Rate** |
| Sample\_I(Normal) | 23,060,971 | 17,648,277 | 1,759,079 | 2,589,570 | 882,839 | 93.83% |
| Sample\_II(Normal) | 22,932,551 | 17,908,040 | 1,392,713 | 2,572,766 | 775,312 | 93.87% |
| Sample\_III(Normal) | 23,202,034 | 18,079,040 | 1,304,704 | 2,623,134 | 755,500 | 93.20% |
| Sample\_I(Stress) | 22,853,565 | 16,992,052 | 1,016,790 | 3,130,632 | 809,474 | 91.61% |
| Sample\_II(Stress) | 22,789,255 | 17,388,698 | 978,048 | 3,073,675 | 772,264 | 93.36% |
| Sample\_III(Stress) | 22,670,166 | 17,378,857 | 1,059,504 | 2,913,333 | 759,543 | 93.16% |

**Table S6. The most enriched KEGG Pathways of local salt tolerant wheat landrace (Roshan).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Enriched Up-regulated KEGG Pathways** | **ID** | **Input number** | **Background number** | **Corrected P-Value** |
| Metabolic pathways | osa01100 | 752 | 1573 | 8.43889095537e-46 |
| Biosynthesis of secondary metabolites | osa01110 | 450 | 848 | 2.23174609307e-34 |
| Peroxisome | osa04146 | 64 | 80 | 1.96056205362e-09 |
| Fatty acid metabolism | osa01212 | 57 | 68 | 5.94848074387e-09 |
| Glycerophospholipid metabolism | osa00564 | 54 | 71 | 1.83468005574e-07 |
| Carbon metabolism | osa01200 | 115 | 235 | 4.45241019011e-07 |
| Glutathione metabolism | osa00480 | 53 | 73 | 6.29100269329e-07 |
| Galactose metabolism | osa00052 | 43 | 53 | 1.47976943432e-06 |
| Alanine, aspartate and glutamate metabolism | osa00250 | 37 | 43 | 2.65790641071e-06 |
| Pyruvate metabolism | osa00620 | 51 | 79 | 7.696634527e-06 |
| Starch and sucrose metabolism | osa00500 | 81 | 159 | 7.83355121104e-06 |
| Endocytosis | osa04144 | 69 | 127 | 8.9965440068e-06 |
| Cysteine and methionine metabolism | osa00270 | 56 | 96 | 1.91377104888e-05 |
| Plant hormone signal transduction | osa04075 | 87 | 184 | 2.36640911779e-05 |
| Lysine degradation | osa00310 | 25 | 25 | 3.09862022017e-05 |
| Biosynthesis of amino acids | osa01230 | 92 | 207 | 6.75657268927e-05 |
| **Enriched Down-regulated KEGG Pathways** | **ID** | **Input number** | **Background number** | **Corrected P-Value** |
| Metabolic pathways | osa01100 | 995 | 1573 | 4.01724652077e-64 |
| Biosynthesis of secondary metabolites | osa01110 | 607 | 848 | 3.60693065928e-50 |
| Ribosome | osa03010 | 228 | 337 | 1.91654791571e-16 |
| Carbon metabolism | osa01200 | 176 | 235 | 1.30415183214e-15 |
| Phenylpropanoid biosynthesis | osa00940 | 106 | 125 | 1.06598175715e-11 |
| Pentose phosphate pathway | osa00030 | 50 | 51 | 6.19543626165e-07 |
| Starch and sucrose metabolism | osa00500 | 97 | 159 | 1.23192853198e-05 |
| Plant hormone signal transduction | osa04075 | 105 | 184 | 4.18563439221e-05 |