

**Comparative Physiological and Metabolic Analysis Reveals a Complex Mechanism Involved in Drought Tolerance in Chickpea (*Cicer arietinum L.*) Induced by PGPR and PGRs**

**Running Title: Physiological and Metabolic Changes Induced by PGPR and PGRs in Chickpea**

Naeem Khan<sup>1</sup>, Asghari Bano<sup>2\*</sup>, M Atikur Rahman<sup>3</sup>, Jia Guo<sup>3</sup>, Zhiyu Kang<sup>4</sup> and Md Ali Babar<sup>3\*</sup>

<sup>1</sup>Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan

<sup>2</sup>Department of Biosciences, University of Wah, Wah Cantt, Pakistan

<sup>3</sup>Department of Agronomy, IFAS, University of Florida, Gainesville, FL, USA

<sup>4</sup>Zhiyu Kang, College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming, Yunnan province (650201), China

Corresponding authors: \*[mababar@ufl.edu](mailto:mababar@ufl.edu) and \*[banoasghari@gmail.com](mailto:banoasghari@gmail.com)

**S1. List of 55 significantly different levels of metabolites (ANOVA,  $P \leq 0.05$ ) with their molecular formula, compound type, p-value, false discovery rate (FDR) and fold change (consortium/control and consortium/drought) in chickpea leaves under control, consortium and drought conditions.**

| S# | Metabolites          | Compound type | Molecular Formula   | SENSITIVE VARIETY (PB-NOOR-2008) |          |                                  |          |           |                                  | TOLERANT VARIETY (93127) |           |                                  |          |           |                                  |
|----|----------------------|---------------|---|----------------------------------|----------|----------------------------------|----------|-----------|----------------------------------|--------------------------|-----------|----------------------------------|----------|-----------|----------------------------------|
|    |                      |               |   | p.value                          | FDR      | Fold Change (Consortium/Control) | p-value  | FDR       | Fold Change (Consortium/Drought) | p.value                  | FDR       | Fold Change (Consortium/Control) | p-value  | FDR       | Fold Change (Consortium/Drought) |
| 1  | Threonine/Homoserine | AMINO ACID    | C <sub>4</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub> | 0.042454                         | 0.045353 | 0.9468                           | 1.64E-05 | 0.0001414 | 1.68094                          | 1.67E-07                 | 0.049582  | 0.99299                          | 1.64E-05 | 0.0001414 | 1.9921                           |
| 2  | L-Asparagine         | AMINO ACID    | C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub> | 0.001829                         | 0.04471  | 1.6521                           | 0.03234  | 0.04037   | 1.138                            | 0.00018936               | 0.0029541 | 1.9976                           | 0.044253 | 0.01443   | 0.55303                          |
| 3  | BOC-D-               | AMINO         | C <sub>14</sub> H <sub>19</sub>                             | 0.002                            | 0.002    | 0.86839                          | 0.000    | 0.002     | 1.1214                           | 0.0382                   | 0.00299   | 1.7882                           | 0.00815  |           | 1.96116                          |

|    |                     |            |            |           |          |          |              |            |         |            |            |         |            |            |         |
|----|---------------------|------------|------------|-----------|----------|----------|--------------|------------|---------|------------|------------|---------|------------|------------|---------|
|    | Phenylalanine       | ACID       | NO4        | 2534      | 854      |          | 876          | 732        |         | 49         |            |         | 46         | 0.03742    |         |
| 4  | Alanine/Sarcosine   | AMINO ACID | C3H7NO2    | 0.042483  | 0.010348 | 1.5029   | 1.81E-05     | 0.00014141 | 0.60541 | 0.00079207 | 0.0077227  | 1.82054 | 1.81E-05   | 0.00014141 | 0.6043  |
| 5  | BOC-L-Tyrosine      | AMINO ACID | C14H19NO5  | 0.00334   | 0.029835 | 1.0076   | 0.00108      | 0.0032084  | 1.4214  | NS         | NS         | NS      | 0.00108    | 0.0032084  | 0.75756 |
| 6  | L-Methionine        | AMINO ACID | C5H11NO2S  | 0.022453  | 0.032457 | 0.86428  | 4.05E-08     | 6.42E-07   | 2.3553  | 4.18E-06   | 0.00010869 | 2.3026  | 4.05E-08   | 6.42E-07   | 5.2437  |
| 7  | Aspartate           | AMINO ACID | C4H7NO4    | 0.025845  | 0.004748 | 1.92148  | 2.67E-07     | 3.47E-06   | 0.31464 | 0.00040921 | 0.0053197  | 3.1182  | 2.67E-07   | 3.47E-06   | 1.22958 |
| 8  | L-Histidine         | AMINO ACID | C6H9NO2    | 0.012467  | 0.045097 | 0.6259   | 5.70E-05     | 0.00034201 | 2.0353  | 0.0014049  | 0.027589   | 0.95862 | 5.70E-05   | 0.00034201 | 1.7102  |
| 9  | 5-OXO-L-Proline     | AMINO ACID | C5H7NO3    | 0.0039265 | 0.028199 | 0.76712  | 0.0021348    | 0.0055505  | 1.9273  | 0.011939   | 0.0055505  | 1.52248 | 0.0021348  | 0.0055505  | 2.3664  |
| 10 | Tryptophan-2,3,3-D3 | AMINO ACID | C11H12N2O2 | 4.87E-07  | 3.85E-05 | 0.038543 | 0.005072     | 0.011635   | 4.91136 | 1.52E-08   | 0.027589   | 2.811   | 0.0012404  | 0.0034555  | 7.2263  |
| 11 | Proline             | AMINO ACID | C5H9NO2    | 0.0016791 | 0.020758 | 0.53456  | 0.033542     | 0.029574   | 4.5811  | 0.0031431  | 0.027589   | 1.15437 | 0.053542   | 0.03177    | 2.2191  |
| 12 | L-Leucine 13C6      | AMINO ACID | C6H13NO2   | NS        | NS       | NS       | 0.00025037   | 0.0011488  | 1.2866  | NS         | NS         | NS      | 0.00025037 | 0.0011488  | 1.0392  |
| 13 | L-Isoleucine        | AMINO ACID | C6H13NO2   | 0.003143  | 0.022875 | 1.10047  | 0.0200131488 | 0.0093853  | 1.9031  | 0.033942   | 0.023862   | 0.774   | 0.003971   | 0.009385   | 1.8007  |

|    |                           |                        |          |           |           |         |            |            |        |           |          |         |            |            |         |
|----|---------------------------|------------------------|----------|-----------|-----------|---------|------------|------------|--------|-----------|----------|---------|------------|------------|---------|
| 14 | 4-Acetamidobutanoate      | AMINO ACID             | C6H10NO3 | 0.0418593 | 0.048256  | 0.9515  | 0.032844   | 0.042534   | 1.7819 | 0.038544  | 0.042954 | 1.0789  | 0.02524    | 0.04262    | 1.7314  |
| 15 | 5-Aminolevulinic Acid     | NON PROTIEN AMINO ACID | C5H9NO3  | 0.03325   | 0.027698  | 1.3586  | 0.022144   | 0.018648   | 1.2418 | 0.024195  | 0.041995 | 1.1983  | 0.026456   | 0.03456    | 0.48264 |
| 16 | Nicotinate                | ORGANIC COMPOUND       | C6H5NO2  | 0.029868  | 0.035132  | 0.76863 | 0.00074612 | 0.0025303  | 1.5513 | 0.012431  | 0.03265  | 0.89901 | 0.00074612 | 0.0025303  | 2.3142  |
| 17 | 4-Hydroxy-L-Phenylglycine | CHEMICAL COMPOUND      | C8H9NO3  | 0.018467  | 0.009573  | 1.8117  | 3.55E-05   | 0.00025173 | 0.4991 | 0.0028267 | 0.031001 | 1.2659  | 3.55E-05   | 0.00025173 | 0.99374 |
| 18 | L--Lactic Acid            | ORGANIC COMPOUND       | C3H6O3   | 0.010375  | 0.045097  | 0.61998 | 0.00715    | 0.014802   | 1.8606 | NS        | NS       | NS      | 0.00715    | 0.014802   | 1.3207  |
| 19 | Phenylpyruvate            | ORGANIC COMPOUND       | C9H8O3   | 0.015042  | 0.049512  | 0.76334 | 0.0034132  | 0.0085882  | 1.3726 | 0.040726  | 0.019322 | 0.8137  | 0.0034132  | 0.0085882  | 1.0965  |
| 20 | Dibutylphthalate          | ORGANIC COMPOUND       | C16H22O4 | NS        | NS        | NS      | 0.0018551  | 0.0049895  | 1.2062 | 0.035493  | 0.049524 | 1.0197  | 0.0018551  | 0.0049895  | 1.0148  |
| 21 | Syringic Acid             | CHEMICAL COMPOUND      | C9H10O5  | NS        | NS        | NS      | 0.04608    | 0.041471   | 1.3422 | 0.041255  | 0.036265 | 0.74377 | NS         | NS         | NS      |
| 22 | Allantoin                 | CHEMICAL               | C4H6N4O3 | 1.92E-05  | 0.0006913 | 0.83083 | 0.020      | 0.042      | 1.771  | NS        | NS       | NS      | 0.00620    |            | 2.3143  |

|    |                               |                   |            |           |          |               |           |          |               |           |           |          |           |           |         |
|----|-------------------------------|-------------------|------------|-----------|----------|---------------|-----------|----------|---------------|-----------|-----------|----------|-----------|-----------|---------|
|    |                               | COMPOUND          |            |           | 3        |               | 082       | 634      |               |           |           |          | 82        | 0.004922  |         |
| 23 | 2-Aminophenol                 | ORGANIC COMPOUND  | C6H7NO     | 0.022424  | 0.043953 | 1.0109        | NS        | NS       | NS            | 0.0093575 | 0.056145  | 0.97942  | 0.041363  | 0.02634   | 1.4987  |
| 24 | Glutarate                     | ORGANIC COMPOUND  | C5H8O4     | 0.032423  | 0.035246 | 0.96281       | 0.019132  | 0.029261 | <b>1.9742</b> | 0.04922   | 0.0.36224 | 1.3571   | 0.019132  | 0.029261  | 1.8798  |
| 25 | Citrulline                    | ORGANIC COMPOUND  | C6H13NO3   | 0.037474  | 0.015942 | 1.3648        | 1.06E-06  | 1.18E-05 | 2.2151        | NS        | NS        | NS       | 1.06E-06  | 1.18E-05  | 2.1002  |
| 26 | 3,5-Di-tert-butylbenzaldehyde | CHEMICAL COMPOUND | C15H22O    | NS        | NS       | NS            | 0.0085414 | 0.01606  | 0.55734       | 0.0082513 | 0.02342   | 2.836    | 0.0085414 | 0.01606   | 1.9226  |
| 27 | D-Saccharic Acid              | CHEMICAL COMPOUND | C6H10O8    | 0.0026276 | 0.020758 | 0.75914       | 0.018537  | 0.029062 | 1.2337        | NS        | NS        | NS       | 0.018537  | 0.029062  | 0.89319 |
| 28 | Triethyl phosphate            | CHEMICAL COMPOUND | C6H15O4P   | 0.038213  | 0.012749 | 0.92564       | 0.0077088 | 0.015418 | 1.3068        | NS        | NS        | NS       | 0.0077088 | 0.015418  | 1.0519  |
| 29 | N-Butylbenzenesulfonamide     | CHEMICAL COMPOUND | C10H15NO2S | 0.012987  | 0.045097 | <b>3.6937</b> | 0.029854  | 0.02433  | 0.13047       | 0.926     | 0.0014376 | 0.027589 | 2.1847    | 0.013047  | 0.16153 |
| 30 | Pipecolate/L-Pipecolic Acid   | ORGANIC MOLECULE  | C6H11NO2   | 0.02537   | 0.003951 | 0.80534       | 0.043969  | 0.0163   | 1.73412       | 0.001507  | 0.013061  | 0.87957  | 0.013969  | 0.0045325 | 2.1193  |

|    |                             |                   |            |           |          |         |            |           |         |          |          |         |            |           |         |
|----|-----------------------------|-------------------|------------|-----------|----------|---------|------------|-----------|---------|----------|----------|---------|------------|-----------|---------|
| 31 | Dopamine                    | ORGANIC CHEMICAL  | C8H11NO2   | 0.004254  | 0.029875 | 1.47561 | 0.04958    | 0.02424   | 0.04532 | 0.012345 | 0.03801  | 1.1155  | 0.005323   | 0.037442  | 0.7977  |
| 32 | Tartaric Acid               | ORGANIC ACID      | C4H6O6     | 0.023958  | 0.048373 | 1.2327  | NS         | NS        | NS      | 0.041879 | 0.02744  | 0.825   | 0.032789   | 0.049223  | 1.0949  |
| 33 | Citrate                     | ORGANIC ACID      | C6H8O7     | 0.034865  | 0.046286 | 0.78882 | NS         | NS        | NS      | 0.014744 | 0.00642  | 1.9457  | 0.007212   | 0.014802  | 1.5867  |
| 34 | Cytidine                    | NUCLEOSIDE        | C9H13N3O5  | 0.0025008 | 0.020758 | 0.55976 | 0.032535   | 0.04745   | 0.99644 | 0.026247 | 0.03993  | 0.59427 | 0.049875   | 0.048785  | 1.0161  |
| 35 | Adenosine                   | NUCLEOSIDE        | C10H13N5O4 | NS        | NS       | NS      | 0.0079924  | 0.015585  | 1.4215  | 0.02768  | 0.044523 | 0.58496 | 0.0079924  | 0.015585  | 0.91276 |
| 36 | Guanine                     | NUCLEOBASE        | C5H5N5O    | 0.013129  | 0.045097 | 1.8827  | 0.006474   | 0.014027  | 2.2203  | NS       | NS       | NS      | 0.0064741  | 0.014027  | 1.0069  |
| 37 | Succinate                   | DICARBOXYLIC ACID | C4H6O4     | 0.023693  | 0.01543  | 0.62089 | 0.012398   | 0.020575  | 2.2754  | 0.03437  | 0.033431 | 0.57205 | 0.012398   | 0.020575  | 1.2616  |
| 38 | 3-Hydroxy-3-Methylglutarate | DICARBOXYLIC ACID | C6H10O5    | 0.011511  | 0.017476 | 1.6331  | 3.49E-09   | 1.20E-07  | 0.58684 | 0.02971  | 0.005242 | 2.1484  | 3.49E-09   | 1.20E-07  | 1.3843  |
| 39 | Glyceric Acid               | SUGAR ACID        | C3H6O4     | 0.02426   | 0.041253 | 0.88825 | 0.0036596  | 0.0089202 | 1.6064  | NS       | NS       | NS      | 0.0036596  | 0.0089202 | 1.6045  |
| 40 | Glucosamine/Mannosamine     | AMINO SUGAR       | C6H13NO5   | 0.01735   | 0.041873 | 2.97119 | 0.00024217 | 0.0011488 | 1.2702  | 0.031245 | 0.025252 | 3.5942  | 0.00024217 | 0.0011488 | 2.1578  |

|    |                         |                       |   |            |          |         |           |           |          |           |           |         |           |          |         |
|----|-------------------------|-----------------------|---|------------|----------|---------|-----------|-----------|----------|-----------|-----------|---------|-----------|----------|---------|
| 41 | Nicotinamide            | VITAMIN               | C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O                | 0.005424   | 0.011293 | 1.6602  | 4.12E-08  | 6.42E-07  | 0.33112  | 0.036834  | 0.095253  | 1.4331  | 4.12E-08  | 6.42E-07 | 0.2246  |
| 42 | Riboflavin              | VITAMIN               | C <sub>17</sub> H <sub>20</sub> N <sub>4</sub> O <sub>6</sub> | 0.011032   | 0.011943 | 1.91284 | 4.63E-09  | 1.20E-07  | 0.033231 | 0.007823  | 0.035477  | 2.8154  | 4.63E-09  | 1.20E-07 | 0.2145  |
| 43 | Ascorbic Acid           | VITAMIN               | C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>                  | 0.022624   | 0.003774 | 1.05779 | 3.98E-06  | 3.88E-05  | 1.51905  | 0.014736  | 0.044238  | 0.41135 | 3.98E-06  | 3.88E-05 | 0.45511 |
| 44 | 4-Coumarate             | ENZYME                | C <sub>18</sub> H <sub>16</sub> O <sub>4</sub>                | NS         | NS       | NS      | 0.038573  | 0.0025834 | 1.195    | NS        | NS        | NS      | 0.044321  | 0.015475 | 1.1891  |
| 45 | Alpha-Ketoglutaric Acid | DERIVATIVE            | C <sub>5</sub> H <sub>6</sub> O <sub>5</sub>                  | 0.043861   | 0.11244  | 1.2488  | NS        | NS        | NS       | 0.02418   | 0.025751  | 0.54993 | 0.036332  | 0.031242 | 1.0591  |
| 46 | N-Methyl-L-Glutamate    | DERIVATIVE            | C <sub>6</sub> H <sub>11</sub> N <sub>1</sub> O <sub>4</sub>  | 0.0064138  | 0.038976 | 3.6462  | 0.0061121 | 0.013621  | 0.53655  | 0.0044003 | 0.034763  | 2.124   | 0.0061121 | 0.013621 | 0.65104 |
| 47 | Erucamide               | FATTY ACID DERIVATIVE | C <sub>22</sub> H <sub>43</sub> NO                            | NS         | NS       | NS      | 0.04736   | 0.04334   | 1.2266   | NS        | NS        | NS      | 0.02736   | 0.03955  | 0.96369 |
| 48 | Isocytosine             | PYRIMIDINE BASE       | C <sub>4</sub> H <sub>5</sub> N <sub>3</sub> O                | 0.0022901  | 0.020758 | 0.55904 | 0.043635  | 0.023467  | 1.0098   | NS        | NS        | NS      | 0.043564  | 0.049852 | 1.0193  |
| 49 | Uridine                 | PYRIMIDINE ANALOG     | C <sub>9</sub> H <sub>12</sub> N <sub>2</sub> O <sub>6</sub>  | 0.0086861  | 0.045097 | 0.61422 | 0.0094668 | 0.017172  | 1.4615   | 0.044183  | 0.032573- | 0.46259 | 0.0094668 | 0.017172 | 1.1186  |
| 50 | Isocitric Acid          | ISOMER                | C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>                  | NS         | NS       | NS      | 0.011934  | 0.020237  | 1.7403   | 0.03964   | 0.043734  | 0.82764 | 0.011934  | 0.020237 | 1.0791  |
| 51 | Glycerol                | POLYOL                | C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>                  | 0.00074328 | 0.011744 | 1.7003  | 0.0002719 | 0.0011784 | 0.46959  | 0.0067165 | 0.047626  | 2.5975  | 0.021339  | 0.032008 | 0.55017 |

|    |                    |                   |            |           |            |         |            |           |        |           |          |        |            |           |         |
|----|--------------------|-------------------|------------|-----------|------------|---------|------------|-----------|--------|-----------|----------|--------|------------|-----------|---------|
|    |                    |                   |            |           |            |         | 4          |           |        |           |          |        |            |           |         |
| 52 | N-Acetylputrescine | POLYAMINE         | C6H14N2O   | 0.0002211 | 0.0043668  | 0.75027 | 0.00073549 | 0.0025303 | 1.4475 | NS        | NS       | NS     | 0.010852   | 0.01881   | 1.6451  |
| 53 | Leu Pro            | PEPTIDE           | C11H20N2O3 | 2.63E-05  | 0.00069133 | 0.45916 | 2.10E-11   | 1.63E-09  | 3.0445 | 0.0020366 | 0.015885 | 1.7947 | 2.10E-11   | 1.63E-09  | 2.3216  |
| 54 | Caffeine-D3        | STIMULANT         | C8H10N4O2  | NS        | NS         | NS      | 0.00031759 | 0.0013038 | 1.2387 | 0.04383   | 0.038353 | 1.0007 | 0.00031759 | 0.0013038 | 0.99975 |
| 55 | L-Carnitine        | AMMONIUM COMPOUND | C7H15NO3   | 0.0043524 | 0.028653   | 0.53596 | 0.018088   | 0.029062  | 1.5294 | NS        | NS       | NS     | 0.018088   | 0.029062  | 0.84622 |

NS- Non-significant