

## **Integrated analysis of Multifactorial Stress Combination impact on citrus plants**

Lledó Rodríguez-Azorín<sup>1</sup>, Aurelio Gómez-Cadenas<sup>1</sup>, María F. López-Climent<sup>1\*</sup>, Vicente Vives-Peris<sup>1\*</sup>.

*<sup>1</sup>Department of Biology, Biochemistry and Natural Sciences, Universitat Jaume I, Avda. Sos Baynat s/n, 12071, Valencian Community, Castelló de la Plana, Castelló, Spain*

\*Corresponding authors: Vicente Vives-Peris and María Fernanda López-Climent; E-mail addresses: [vvives@uji.es](mailto:vvives@uji.es) and [mcliment@uji.es](mailto:mcliment@uji.es). Phone numbers: +34 964728101 and +34 964729414

Journal: Planta

**Online resource S1.** Irrigation solutions composition at 10X concentration. CT = control condition; N = nitrogen deficiency condition; P = phosphorus deficiency condition; K = potassium deficiency condition; combined letters = combination of different nutritional deficiencies.

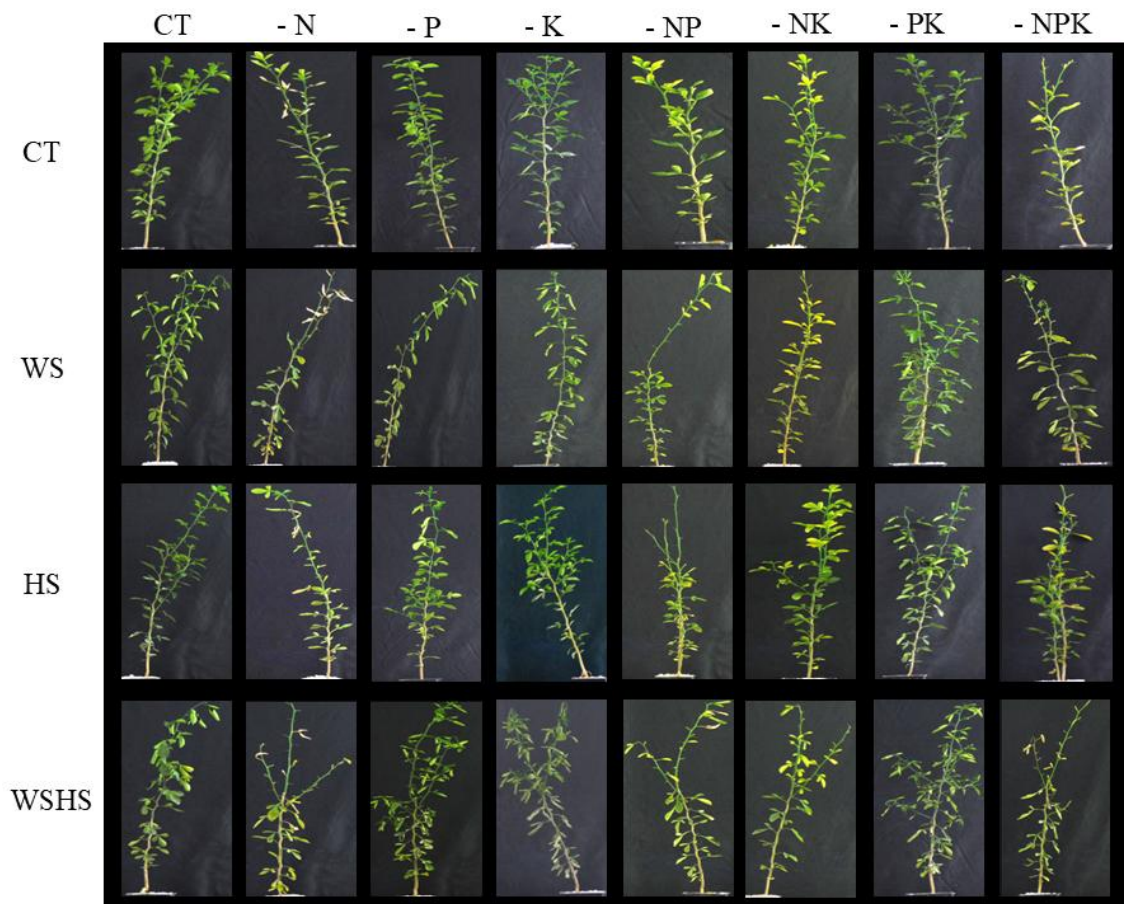
| Irrigation solution 10X |                                                 |       |
|-------------------------|-------------------------------------------------|-------|
| Condition               | Reagents                                        | (g/L) |
| CT                      | KNO <sub>3</sub>                                | 40    |
|                         | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | H <sub>3</sub> PO <sub>4</sub> 72%              | 9.3   |
|                         | Nutrishell                                      | 4     |
|                         | Ca(NO <sub>3</sub> ) <sub>2</sub>               | 82    |
| N                       | K <sub>2</sub> SO <sub>4</sub>                  | 34.48 |
|                         | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | H <sub>3</sub> PO <sub>4</sub> 72%              | 9.3   |
|                         | Nutrishell                                      | 4     |
|                         | CaCl <sub>2</sub>                               | 55.4  |
| P                       | KNO <sub>3</sub>                                | 40    |
|                         | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | Nutrishell                                      | 4     |
|                         | Ca(NO <sub>3</sub> ) <sub>2</sub>               | 82    |
| K                       | (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 26.2  |
|                         | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | H <sub>3</sub> PO <sub>4</sub> 72%              | 9,3   |
|                         | Nutrishell                                      | 4     |
|                         | Ca(NO <sub>3</sub> ) <sub>2</sub>               | 82    |
| NK                      | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | H <sub>3</sub> PO <sub>4</sub> 72%              | 9,3   |
|                         | Nutrishell                                      | 4     |
|                         | CaCl <sub>2</sub>                               | 55.4  |
| NP                      | K <sub>2</sub> SO <sub>4</sub>                  | 34.48 |
|                         | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | Nutrishell                                      | 4     |
|                         | CaCl <sub>2</sub>                               | 55.4  |
| PK                      | K <sub>2</sub> SO <sub>4</sub>                  | 34.48 |
|                         | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | Nutrishell                                      | 4     |
|                         | Ca(NO <sub>3</sub> ) <sub>2</sub>               | 82    |
| NPK                     | MgSO <sub>4</sub> · 7 H <sub>2</sub> O          | 40    |
|                         | Nutrishell                                      | 4     |
|                         | CaCl <sub>2</sub>                               | 55.4  |

**Online resource S2.** The thirty-two combinations based on the applied abiotic stress combination. CT = control condition; N = nitrogen deficiency condition; P = phosphorus deficiency condition; K = potassium deficiency condition; WS = water stress condition; HS = heat stress condition; combined letters = combined stresses.

| Combinations<br>1 – 4           |                                 | Combinations<br>5 – 8           |                                 | Combinations<br>9 – 12          |                                 | Combinations<br>13 – 16         |                                 |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress | 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress | 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress | 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress |
| <b>CT</b>                       | CT                              | <b>N</b>                        | CT                              | <b>P</b>                        | CT                              | <b>K</b>                        | CT                              |
|                                 | WS                              |                                 | WS                              |                                 | WS                              |                                 | WS                              |
|                                 | HS                              |                                 | HS                              |                                 | HS                              |                                 | HS                              |
|                                 | WSHS                            |                                 | WSHS                            |                                 | WSHS                            |                                 | WSHS                            |
| Combinations<br>17 – 20         |                                 | Combinations<br>21 – 24         |                                 | Combinations<br>25 – 28         |                                 | Combinations<br>29 – 32         |                                 |
| 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress | 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress | 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress | 1 <sup>st</sup> phase<br>stress | 2 <sup>nd</sup> phase<br>stress |
| <b>NP</b>                       | CT                              | <b>NK</b>                       | CT                              | <b>PK</b>                       | CT                              | <b>NPK</b>                      | CT                              |
|                                 | WS                              |                                 | WS                              |                                 | WS                              |                                 | WS                              |
|                                 | HS                              |                                 | HS                              |                                 | HS                              |                                 | HS                              |
|                                 | WSHS                            |                                 | WSHS                            |                                 | WSHS                            |                                 | WSHS                            |



**Online Resource S3.** Standard on which leaf damage level was based.



**Online Resource S4.** Picture of the most representative citrus plant exposed to a stress combination on the sampling day. In the horizontal axis, abbreviations of the first phase groups: CT = control; N = nitrogen deficiency; P = phosphorous deficiency; K = potassium deficiency; combine letters = combined deficiencies. In the vertical axis, abbreviations of the second phase groups: CT = control group; WS =water stress group; HS = heat stress group; combined letters = combined stresses.

**Online resource S5.** Chlorophyll A (ChlA) and chlorophyll B (ChlB) content depending on the number of stresses in plants subjected to Multifactorial Stress Combination. Data refer to mean values  $\pm$  SE. Statistical analysis was performed by ANOVA test using  $P$ -value = 0.05 comparing all the groups simultaneously. Different letters denote significative difference between groups in “ANOVA” column. An additional statistical analysis was performed by Student’s t-test using  $P$ -value = 0.05. Ref. = reference.

| Stresses number | Chl a content               |        |       |           | Chl b content               |        |       |           |
|-----------------|-----------------------------|--------|-------|-----------|-----------------------------|--------|-------|-----------|
|                 | Mean ( $\mu\text{g/g FW}$ ) | SE     | ANOVA | T-student | Mean ( $\mu\text{g/g FW}$ ) | SE     | ANOVA | T-student |
| 0               | 1803.50                     | 40.96  | a     | Ref.      | 575.35                      | 14.78  | a     | Ref.      |
| 1               | 1765.97                     | 92.96  | a     | 0.8627    | 615.46                      | 33.36  | a     | 0.6079    |
| 2               | 1733.40                     | 96.56  | a     | 0.8225    | 628.44                      | 38.24  | a     | 0.6683    |
| 3               | 1619.45                     | 114.84 | a     | 0.6210    | 607.89                      | 52.82  | a     | 0.8490    |
| 4               | 1626.49                     | 148.56 | a     | 0.6105    | 583.44                      | 59.43  | a     | 0.9534    |
| 5               | 1496.38                     | 76.11  | a     | 0.0253    | 530.50                      | 330.98 | a     | 0.2614    |

**Online Resource S6.** Phytohormone content values of citrus exposed to Multifactorial Stress Combination depending on the number of applied stresses. ABA = abscisic acid; PA = phaseic acid; SA= salicylic acid; IAA = indole acetic acid; JA = jasmonic acid; EE = standard deviation. Data refer to mean values  $\pm$  SE. Statistical analysis was performed by ANOVA test using *P*-value = 0.05 comparing the six groups simultaneously for each hormone. Different letters denote significative difference between groups in “ANOVA” column. An additional statistical analysis was performed by Student’s t-test using *P*-value = 0.05. Ref. = reference.

| Hormone | Stresses number | Mean (ng/g FW) | SE     | ANOVA | T-student |
|---------|-----------------|----------------|--------|-------|-----------|
| ABA     | 0               | 19.66          | 1.74   | a     | Ref.      |
|         | 1               | 159.01         | 43.47  | a     | 0.181     |
|         | 2               | 287.05         | 56.72  | a     | 0.146     |
|         | 3               | 296.58         | 53.62  | a     | 0.112     |
|         | 4               | 252.47         | 58.05  | a     | 0.099     |
|         | 5               | 221.70         | 11.72  | a     | 0.000     |
| PA      | 0               | 92.38          | 6.29   | a     | Ref.      |
|         | 1               | 217.59         | 37.18  | a     | 0.161     |
|         | 2               | 294.07         | 46.46  | a     | 0.179     |
|         | 3               | 323.06         | 48.65  | a     | 0.150     |
|         | 4               | 279.77         | 62.47  | a     | 0.209     |
|         | 5               | 366.69         | 48.49  | a     | 0.005     |
| SA      | 0               | 67.30          | 7.85   | a     | Ref.      |
|         | 1               | 53.10          | 5.12   | a     | 0.235     |
|         | 2               | 58.06          | 3.62   | a     | 0.411     |
|         | 3               | 67.96          | 5.38   | a     | 0.968     |
|         | 4               | 90.31          | 5.43   | a     | 0.077     |
|         | 5               | 119.97         | 12.19  | a     | 0.022     |
| IAA     | 0               | 2.46           | 1.48   | a     | Ref.      |
|         | 1               | 8.14           | 1.09   | a     | 0.037     |
|         | 2               | 10.98          | 1.81   | a     | 0.135     |
|         | 3               | 11.55          | 1.77   | a     | 0.104     |
|         | 4               | 13.84          | 2.55   | a     | 0.071     |
|         | 5               | 7.56           | 3.20   | a     | 0.222     |
| JA      | 0               | 31.02          | 1.74   | a     | Ref.      |
|         | 1               | 43.82          | 12.19  | a     | 0.643     |
|         | 2               | 145.21         | 54.55  | a     | 0.505     |
|         | 3               | 241.47         | 88.17  | a     | 0.455     |
|         | 4               | 576.72         | 297.71 | a     | 0.439     |
|         | 5               | 246.26         | 23.78  | a     | 0.001     |

**Online Resource S7.** Principal Component Report obtained in PCA performed to analyse the differences on leaf damage, total carotenoids and total chlorophylls content, shoot fresh weight,  $\Phi$ PSII, gs, E, MDA content, antioxidant capacity, ABA content, JA content, SA content, PA content and IAA content of citrus plants exposed to Multifactorial Stress Combination depending on the number of stresses (**Fig. 6**).

|                              | <b>PC1</b> | <b>PC2</b> |
|------------------------------|------------|------------|
| <b><math>\Phi</math>PSII</b> | -0.195     | 0.185      |
| <b>E</b>                     | 0.251      | 0.409      |
| <b>gs</b>                    | 0.248      | 0.410      |
| <b>Leaf damage</b>           | 0.0615     | -0.480     |
| <b>Shoot fresh weight</b>    | -0.0231    | 0.376      |
| <b>Total carotenoids</b>     | 0.439      | -0.0371    |
| <b>Total chl</b>             | 0.357      | 0.0605     |
| <b>ABA</b>                   | 0.382      | -0.101     |
| <b>JA</b>                    | 0.383      | -0.0216    |
| <b>SA</b>                    | 0.113      | 0.212      |
| <b>IAA</b>                   | 0.184      | -0.350     |
| <b>PA</b>                    | 0.0525     | -0.246     |
| <b>MDA</b>                   | 0.156      | 0.0513     |
| <b>Antioxidant capacity</b>  | 0.383      | -0.111     |