**Drought stress promotes the colonization success of a herbivorous mite that manipulates plant defences**

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# Supplementary data

**Supplementary Table S1.** Summary of analytical methods to assess the inhibitory activity of plant protein extracts [1]

| Commercial enzyme [2] | Substrate [3] | Buffer [4] | Incubation | Measurement [5] |
|---|---|---|---|---|
| Cathepsin B from bovine spleen (EC 3.4.22.1) | Z-RR-AMC | 100 mM NA phosphate, pH 6.0 (10 mM L-cysteine, 10 mM EDTA, 0.01% (v/v) Brij 35) | 1 h at 28 ºC | excitation filter 350 nm emission filter 465 nm |
| Papain from *Carica papaya* (EC 3.4.22.2) | Z-FR-AMC | 100 mM Na phosphate, pH 6.0 (10 mM L-cysteine, 10 mM EDTA, 0.01% (v/v) Brij 35) | 1 h at 28 ºC | excitation filter 350 nm emission filter 465 nm |
| Cathepsin D from bovine spleen (EC 3.4.23.5) | MocAc-GKPILFFRLK (Dnp)-D-R-NH2 | 100 mM sodium citrate, pH 3.5 (0.15M NaCl, 5 mM MgCl2) | 10 min at 30 ºC | excitation filter 328 nm emission filter 393 nm |
| Trypsin from bovine pancreas (EC 3.4.21.4), | Z-LA-AMC | 100 mM Tris-HCl, pH 7.5 (0.15M NaCl, 5 mM MgCl2) | 1 h at 35 ºC | excitation filter 350 nm emission filter 465 nm |
| α-Chymotrypsin from bovine pancreas (EC 3.4.21.1), | SucAAPF-AMC | 100 mM Tris-HCl, pH 7.5 (0.15M NaCl, 5 mM MgCl2) | 1 h at 35 ºC | excitation filter 350 nm emission filter 465 nm |
| Leucine aminopeptidase from porcine pancreas (EC 3.4.11.1). | LpNa | 100 mM Tris-HCl, pH 8 (0.15M NaCl, 5 mM MgCl2) | 1 h at 30 ºC | absorbance at 410 nm |

[1] Procedures adapted from Ximénez-Embún et al. (2016). Samples of 20 µg of plant protein extracts (40 µg in case of leucine aminopeptidase) were preincubated for 10 min with 100 ng of the commercial enzyme.

[2] All purchased from Sigma-Aldricht (St Luis, USA).

[3] The substrates were added at a final concentration of 20 µM. Z-RR-AMC (N-carbobenzoxyloxy-Arg-Arg-7-amido-4-methylcoumarin) for cathepsin B, Z-FR-AMC (N-carbobenzoxyloxy-Phe-Arg-7-amido-4-methylcoumarin) for papain, Z-LA-AMC (Z-L-Arg-7-amido-4-methylcoumarin) for trypsin, SucAAPF-AMC (Suc-Ala-Ala-Pro-Phe-7-amido-4-methylcoumarin) for chymotrypsin, all purchased from Calbiochem (MerkMilipore, Billerica, USA), MocAc-GKPILFFRLK(Dnp)-D-R-NH2 from Peptanova (Germany) for cathepsin D, and LpNa (L-leucine p-nitroanilide) from Sigma-Aldricht (St Luis, USA) for leucine aminopeptidase.

[4] Concentrations are expressed at molarity in the reaction mixture.

[5] AMC (7-amino-4-methylcoumarin) (Bachem, Swizerland) as standard for all fluorescent substrates, except MCA (MoCAC-Pro-Leu-Gly) (Peptanova GmbH, Germany) for cathepsin D. Double blanks were used to account for spontaneous breakdown of substrates and the plant protease activity, and all assays were done in duplicate.

**Supplementary Table S2** Parameters used for detection of phytohormones and related compounds by LC-MS/MS

| Compound | Molecular ion [M-H] (*m/z*) | Fragment ion (*m/z*) |
|---|---|---|
| **OPDA** | 291 | 165 |
| **JA** | 209 | 59 |
| **$D_5$-JA (Internal Standard)** | 213 | 61 |
| **JA-Ile** | 322 | 130 |
| **SA** | 137 | 93 |
| **$D_6$-SA (Internal Standard)** | 141 | 97 |
| **ABA** | 263 | 153 |

**Supplementary Table S3** Nucleotide sequence of primers used for qRT-PCR analysis

| Target Gene | Name | GenBank (GB) accession | Gen Model ITAG2.3: | Forward Primer 5' → 3' | Reverse Primer 5' → 3' |
|---|---|---|---|---|---|
| ***PPO-F*** | *Polyphenol-oxidase-F* | AK247126.1 | Solyc08g074630.1.1 | CGGAGTTTGCAGGGAGTTATAC | TTGATCTCCACACTTTCAATGG |
| ***JIP-21*** | *Jasmonate-inducible protein 21* | AJ295638.1 | Solyc03g098790.1.1 | ACTCGTCCTGTGCTTTGTCC | CCCAAGAGGATTTTCGTTGA |
| ***TD2*** | *Threonine Deaminase-2* | M61915.1 | Solyc09g008670.2.1 | TGCCGTTAAAAATGTCACCA | ACTGGCGATGCCAAAATATC |
| ***PI-IIf*** | *Proteinase Inhibitor IIf* | AY129402.1 | Solyc03g020080.2.1 | GACAAGGTACTAGTAATCAATTATCC | GGGCATATCCCGAACCCAAGA |
| ***PR-P6*** | *Pathogenesis-related protein P6* | M69248.1 | Solyc00g174340.1.1 | GTACTGCATCTTCTTGTTTCCA | TAGATAAGTGCTTGATGTCCA |
| ***Actin*** | *Actin* | XM_004235020.1 | Solyc03g078400.2.1 | TTAGCACCTTCCAGCAGATGT | AACAGACAGGACACTCGCACT |

**Supplementary Table S4** Results of the three-way ANOVA analysis.

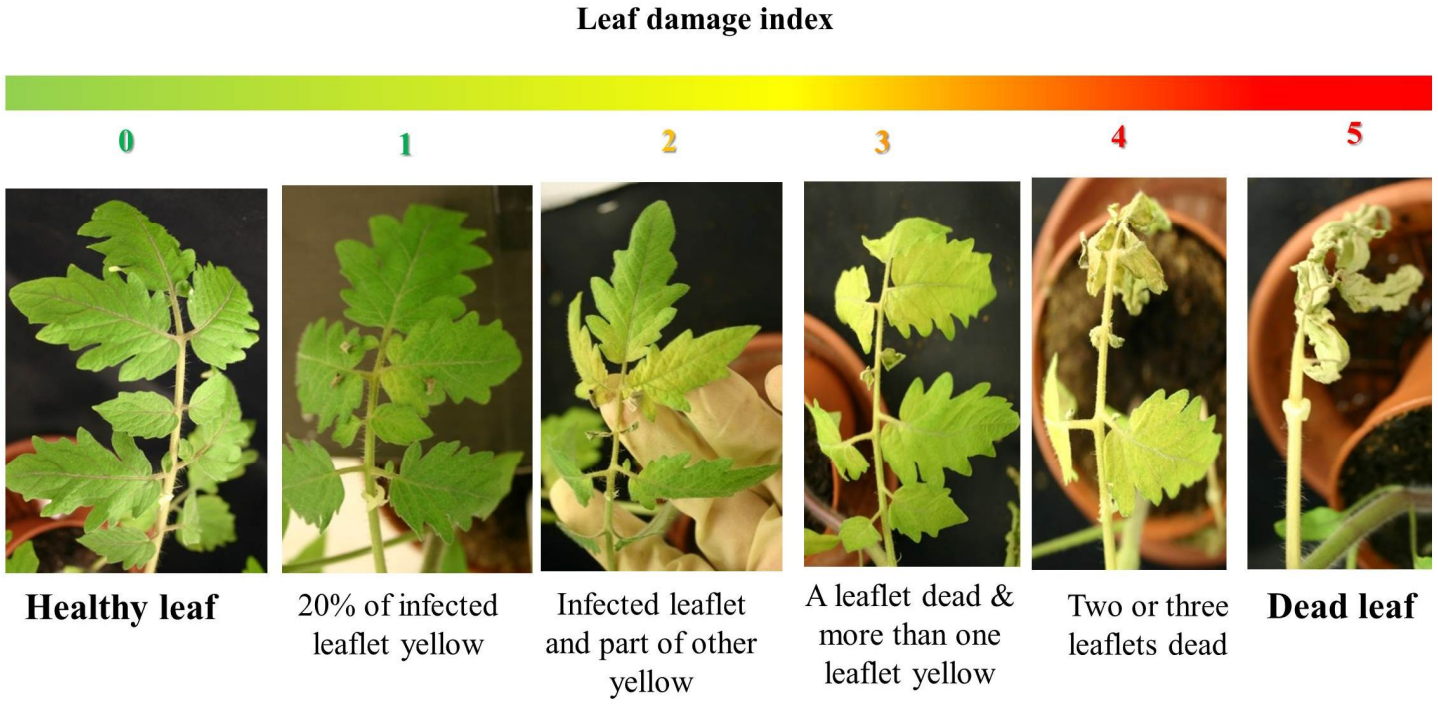
| | Drought | | | TRM | | | Time | | | Drought*TRM[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **F** | **df** | ***p*** | **F** | **df** | ***p*** | **F** | **df** | ***p*** | **F** | **df** | ***p*** |
| **Mite population growth experiment** | | | | | | | | | | | | |
| **Stomatal conductance** | 35.6 | 1,57 | **<0.001** | 4.09 | 1,57 | 0.06 | 421 | 2,57 | **<0.001** | 0.01 | 1,57 | 0.947 |
| **Stem length** | 10.6 | 1,57 | **<0.001** | 0.023 | 1,57 | 0.876 | 12.9 | 1,57 | **<0.001** | 0.31 | 1,57 | 0.578 |
| **Plant sampling and plant damage evaluation experiment** | | | | | | | | | | | | |
| **Stomatal conductance** | 4.42 | 1,55 | **<0.001** | 0.965 | 1,55 | 0.387 | 18.1 | 1,55 | **<0.001** | 2.10 | 1,55 | 0.153 |
| **Stem length** | 193 | 1,55 | **<0.001** | 1.382 | 1,55 | 0.246 | 290 | 1,55 | **<0.001** | 8.30 | 1,55 | **0.006** |

[a]From the different possible interactions between factors only the one studied is presented

**Supplementary Figure S5** Results of the two-way ANOVA analysis.

| | Moderate drought | | | TRM | | | Drought*TRM | | |
|---|---|---|---|---|---|---|---|---|---|
| | **F** | **df** | ***p*** | **F** | **df** | ***p*** | **F** | **df** | ***p*** |
| **Nutrients** | | | | | | | | | |
| **Free sugars** | 10.35 | 1,20 | **0.004** | 1.275 | 1,20 | 0.272 | 5.915 | 1,20 | **0.024** |
| **Protein** | 0.139 | 1,20 | 0.714 | 20.47 | 1,20 | **>0.001** | 0.148 | 1,20 | 0.705 |
| **Total free aa** | 5.074 | 1,20 | **0.036** | 0.66 | 1,20 | 0.426 | 0.229 | 1,20 | 0.642 |
| **Non-essential amino acids** | | | | | | | | | |
| **Asp** | 13.020 | 1,20 | **0.002** | 0.450 | 1,20 | 0.510 | 0.006 | 1,20 | 0.940 |
| **Thr** | 0.461 | 1,20 | 0.505 | 2.676 | 1,20 | 0.117 | 0.293 | 1,20 | 0.594 |
| **Ser** | 12.927 | 1,20 | **0.002** | 2.510 | 1,20 | 0.129 | 0.004 | 1,20 | 0.949 |
| **Glu** | 0.839 | 1,20 | 0.371 | 0.927 | 1,20 | 0.347 | 1.965 | 1,20 | 0.176 |
| **Gly** | 4.041 | 1,20 | 0.058 | 3.643 | 1,20 | 0.071 | 0.024 | 1,20 | 0.878 |
| **Ala** | 19.703 | 1,20 | **0.000** | 1.274 | 1,20 | 0.272 | 0.354 | 1,20 | 0.558 |
| **Cys** | 1.978 | 1,20 | 0.175 | 9.331 | 1,20 | **0.006** | 0.008 | 1,20 | 0.929 |
| **Pro** | 0.976 | 1,20 | 0.335 | 9.218 | 1,20 | **0.007** | 0.524 | 1,20 | 0.477 |
| **Essential amino acids** | | | | | | | | | |
| **Val** | 0.022 | 1,20 | 0.882 | 18.315 | 1,20 | **0.000** | 0.04 | 1,20 | 0.844 |
| **Met** | 3.219 | 1,20 | 0.088 | 3.201 | 1,20 | 0.089 | 0.924 | 1,20 | 0.348 |
| **Ile** | 3.555 | 1,20 | 0.074 | 14.763 | 1,20 | **0.001** | 0.372 | 1,20 | 0.549 |
| **Leu** | 6.472 | 1,20 | **0.019** | 39.416 | 1,20 | **0.000** | 0.038 | 1,20 | 0.848 |
| **Tyr** | 9.716 | 1,20 | **0.005** | 71.310 | 1,20 | **0.000** | 1.599 | 1,20 | 0.221 |
| **Phe** | 2.436 | 1,20 | 0.134 | 3.545 | 1,20 | 0.074 | 0.305 | 1,20 | 0.587 |
| **His** | 5.342 | 1,20 | **0.032** | 1.289 | 1,20 | 0.270 | 3.358 | 1,20 | 0.082 |
| **lys** | 1.683 | 1,20 | 0.209 | 17.287 | 1,20 | **0.000** | 0.71 | 1,20 | 0.410 |
| **Arg** | 4.809 | 1,20 | **0.040** | 26.030 | 1,20 | **0.000** | 0.24 | 1,20 | 0.630 |
| **Phytohormones** | | | | | | | | | |
| **OPDA** | 0.026 | 1,39 | 0.871 | 36 | 1,39 | **<0.001** | 3.560 | 1,39 | 0.067 |
| **JA** | 6.02 | 1,39 | **0.019** | 9.812 | 1,39 | **0.003** | 0.219 | 1,39 | 0.642 |
| **JA-Ile** | 0.955 | 1,39 | 0.334 | 10.76 | 1,39 | **0.002** | 0.565 | 1,39 | 0.457 |
| **ABA** | 8.04 | 1,39 | **0.007** | 0.195 | 1,39 | 0.661 | 0.228 | 1,39 | 0.636 |
| **SA** | 12.48 | 1,39 | **0.001** | 95.88 | 1,39 | **<0.001** | 8.941 | 1,39 | **0.005** |
| **Gene expresion** | | | | | | | | | |
| ***TD-II*** | 3.330 | 1,16 | 0.087 | 11.14 | 1,16 | **0.004** | 1.160 | 1,16 | 0.297 |
| ***PPO-F*** | 7.566 | 1,15 | **0.015** | 4.965 | 1,15 | **0.042** | 1.887 | 1,15 | 0.120 |
| ***JIP-21*** | 5.200 | 1,16 | **0.036** | 5.203 | 1,16 | **0.036** | 0.288 | 1,16 | 0.599 |
| ***PI-IIf*** | 0.208 | 1,16 | 0.654 | 0.273 | 1,16 | 0.608 | 0.005 | 1,16 | 0.943 |
| ***PR-P6*** | 1.543 | 1,16 | 0.232 | 41.24 | 1,16 | **<0.001** | 1.054 | 1,16 | 0.320 |
| **Defense proteins** | | | | | | | | | |
| **Cathepsin B** | 0.209 | 1,20 | 0.653 | 144.1 | 1,20 | **<0.001** | 4.386 | 1,20 | 0.0499 |
| **Papain** | 3.481 | 1,20 | 0.0776 | 42.3 | 1,20 | **<0.001** | 2.632 | 1,20 | 0.1212 |
| **Cathepsin D** | 1.708 | 1,20 | 0.2069 | 1.1 | 1,20 | 0.302 | 0.104 | 1,20 | 0.7507 |
| **Trypsin** | 5.673 | 1,20 | **0.028** | 2.8 | 1,20 | 0.113 | 0.021 | 1,20 | 0.8873 |
| **Chymotrypsin** | 23.54 | 1,20 | **<0.001** | 6.1 | 1,20 | **0.023** | 1.904 | 1,20 | 0.1836 |
| **Aminopeptidase** | 0.059 | 1,20 | 0.8114 | 2.3 | 1,20 | 0.149 | 1.398 | 1,20 | 0.2517 |
| **Polyphenol oxidases** | 8.393 | 1,20 | **0.009** | 3.756 | 1,20 | 0.0676 | 0.664 | 1,20 | 0.4254 |
| **Peroxidases** | 16.46 | 1,20 | **<0.001** | 29.38 | 1,20 | **<0.001** | 0.014 | 1,20 | 0.9082 |
| | **F** | **df** | ***p*** | **F** | **df** | ***p*** | **F** | **df** | ***p*** |
| | **Drought** | | | **TRM** | | | **Drought*TRM** | | |

**Supplementary Figure S1** Leaf damage index explanation



**Supplementary Figure S2** Effect of moderate drought on A) tomato stomatal conductance (gs) and B) stem length at mite infestation (mi) at 7 and 14 days post infestation (dpi). Data (mean ± SE) are average of the values on infested and non-infested w.t. plants on TRM population growth and plant material experiments, as mite infestation didn´t show a significant effect. An asterisk indicates a significant difference between drought and control treatments at each time (Three-way ANOVA, Bonferroni *post hoc* test, $p<0.05$).