

SUPPLEMENTARY MATERIAL

***Daphnia magna* multigeneration exposure to carbendazim: gene transcription responses**

Ana Rita R. Silva^a, Patrícia V. Silva^a, Ana Raquel Soares^b, M. Nazaret González-Alcaraz^c,
Cornelis A.M. van Gestel^d, Dick Roelofs^e, Gabriela Moura^b, Amadeu M. V. M. Soares^a
and Susana Loureiro^a

^aDepartment of Biology & CESAM, University of Aveiro, 3810-193, Portugal

^bDepartment of Medical Sciences & Institute for Biomedicine (iBiMED), University of Aveiro, 3810-193
Aveiro, Portugal

^cDepartment of Agricultural Engineering of the E.T.S.I.A. & Soil Ecology and Biotechnology Unit of the
Institute of Plant Biotechnology, Technical University of Cartagena, 30203, Cartagena, Spain

^dDepartment of Ecological Science, Faculty of Science, Vrije Universiteit Amsterdam, The Netherlands

^eKeygene N.V., Agro Business Park 90, 6708 PW Wageningen, The Netherlands

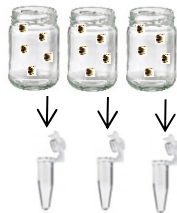
Daphnids in clean medium (Control)

1) Multigenerational experiment (F0 to F12): 1 L glass vessels, 3 replicates, 20 *Daphnia magna* each.



At F0 and F12, *D. magna* neonates (<24h) were randomly collected from these 3 vessels and moved to new vessels:

2) Gene transcription analysis (F0 and F12): 150 mL glass vessels, 3 replicates, 5 *D. magna* each.



After 10 days, the 5 daphnids were pooled together per replicate - shock-frozen in liquid nitrogen and stored at -80°C until RNA extraction.

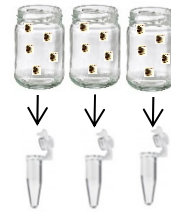
Daphnids in carbendazim (5 $\mu\text{g L}^{-1}$)

1) Multigenerational experiment (F0 to F12): 1 L glass vessels, 3 replicates, 20 *Daphnia magna* each.



At F0 and F12, *D. magna* neonates (<24h) were randomly collected from these 3 vessels and moved to new vessels:

2) Gene transcription analysis (F0 and F12): 150 mL glass vessels, 3 replicates, 5 *D. magna* each.



After 10 days, the 5 daphnids were pooled together per replicate - shock-frozen in liquid nitrogen and stored at -80°C until RNA extraction.

Figure S1. Details of the experimental design of the 1) multigenerational experiment and 2) gene transcription analysis. F represents generation. Note: when moved to new vessels, daphnids were maintained in the same conditions (clean daphnids in clean medium; carbendazim daphnids in carbendazim).

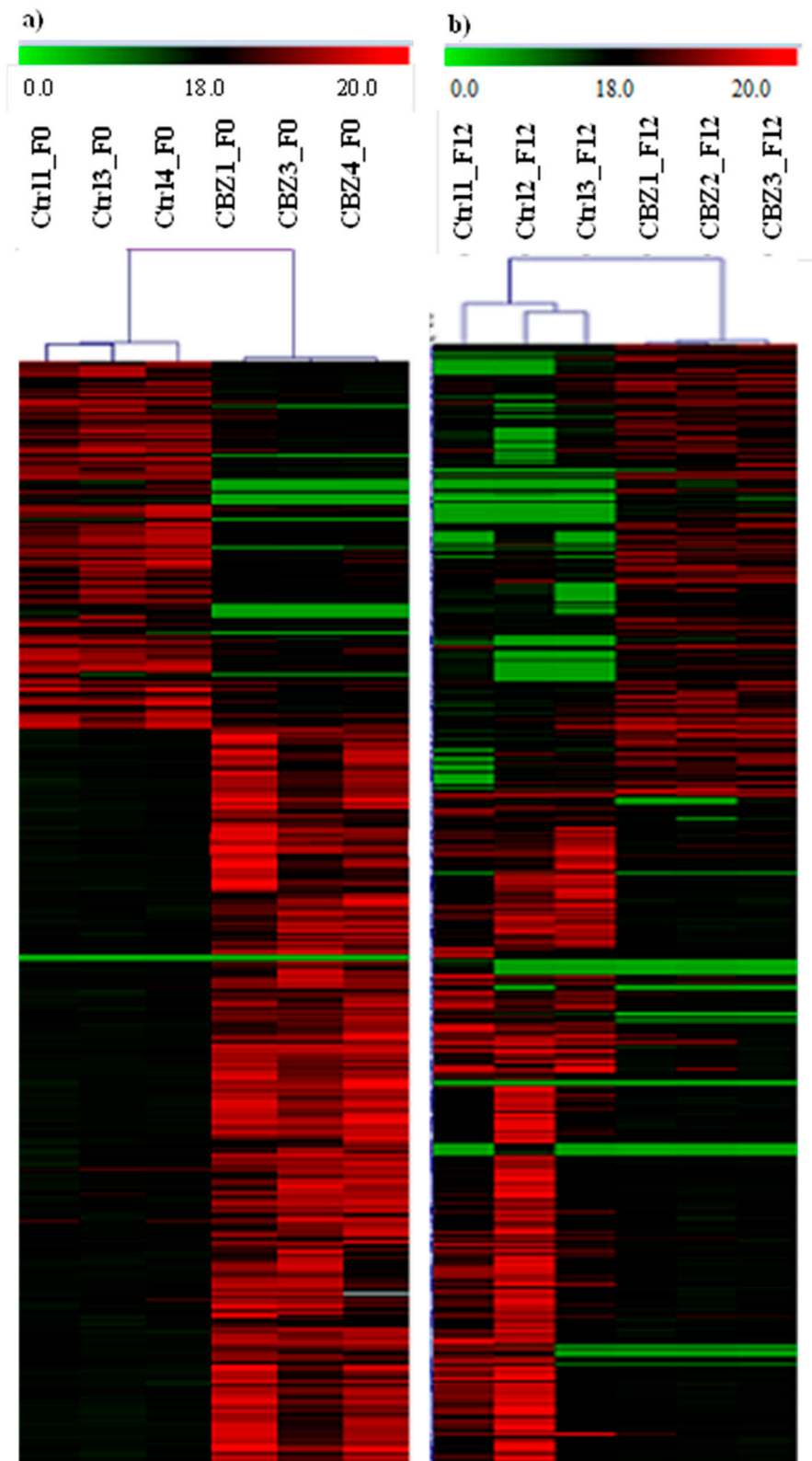


Figure S2. Hierarchical clustering of statistically significantly expressed genes of *Daphnia magna* exposed to 5 $\mu\text{g L}^{-1}$ carbendazim (CBZ) compared with clean medium (controls, Ctrl): **a)** F0 generation, and **b)** F12 generation. A clear separation and clustering between clean medium conditions and carbendazim exposure is observed, with green shading indicating less expressed genes and red shading indicating highly expressed genes.