

File_S1 Global transcriptional analysis suggests *Lasiodiplodia theobromae* pathogenicity factors involved in modulation of grapevine defensive response

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RNA extraction from infected grapevine shoots

Total RNA from *L. theobromae*-infected grapevine was isolated using a cetyltrimethylammonium bromide (CTAB)-based extraction method (Gambino et al., 2008). Briefly, a day previous to RNA extraction, the transversal sections (around 5 mm of length and 100 mg of weight) of infected grapevines was removed from NAP solution and placed in 2.0 mL tubes, containing one ¼ inch ceramic bead and 50 mg of 0.5 mm and 100 mg of 1 mm glass beads (Biospec). Tubes were beaten on a Mini-Beadbeater (Biospec Products) for 30 s. Immediately, tubes were chilled on ice, and pre-heated lysis solution was added (2% v/v CTAB, 2% v/v PVP, Tris-HCl 100 mM pH 8.0, NaCl 2 M, EDTA 25 mM, and 2% of β -mercaptoethanol added just before use) before subjecting the tubes to a second beating cycle for 30 s. Lysed samples were incubated at 65 °C for 5 min and 500 μ l of CIA (24:1 v:v) was added and mixed twice in a Vortex. Tubes were centrifuged at 4,000 rpm for 30 min at 4 °C. The washing with CIA was repeated and then the aqueous phase was transferred to a new 1.5 mL tube and mixed with 1 v of 75% ethanol v:v. The total mix was passed through a silica column (Epochlab) and centrifuged at maximal velocity for 1 min at room temperature. Thereafter, the column was washed with RPE buffer (Qiagen) and twice with ethanol 75% v:v. Finally, total RNA was eluted in 48 μ L of nuclease-free water (Qiagen) by centrifugation at maximal velocity.

References

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