

# **NADPH oxidases Nox1 and Nox2 differentially regulate volatile organic compounds, fungistatic activity, plant growth promotion and nutrients assimilation in *Trichoderma atroviride***

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**Running title:** VOCs and nutrient assimilation are differentially regulated by NADPH oxidases in *Trichoderma*

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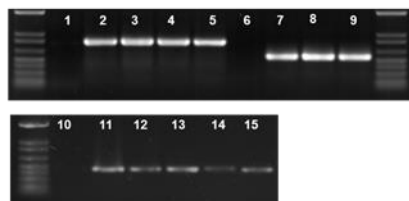
## **Keywords**

*Trichoderma*, plant growth promotion, volatile organic compounds, Nox1, Nox2, NoxR, reactive oxygen species

**Supplementary Figure S1.** A. PCR for confirming the integration of the complementation construct into the  $\Delta$ Nox1,  $\Delta$ Nox2 and  $\Delta$ NoxR deletion mutant strains. PCR from independent transformants was conducted using internal primers which amplify the open reading frame of the genes integrated in the deletion mutants. B. assay of mechanical injury in Nox1 and NoxR and the corresponding complementing strains to demonstrate the recovery of one of the phenotypes of the lack of these genes in *T. atroviride*.

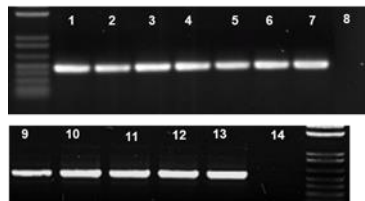
A

A) PCR using primers to amplify the Noxs orfs



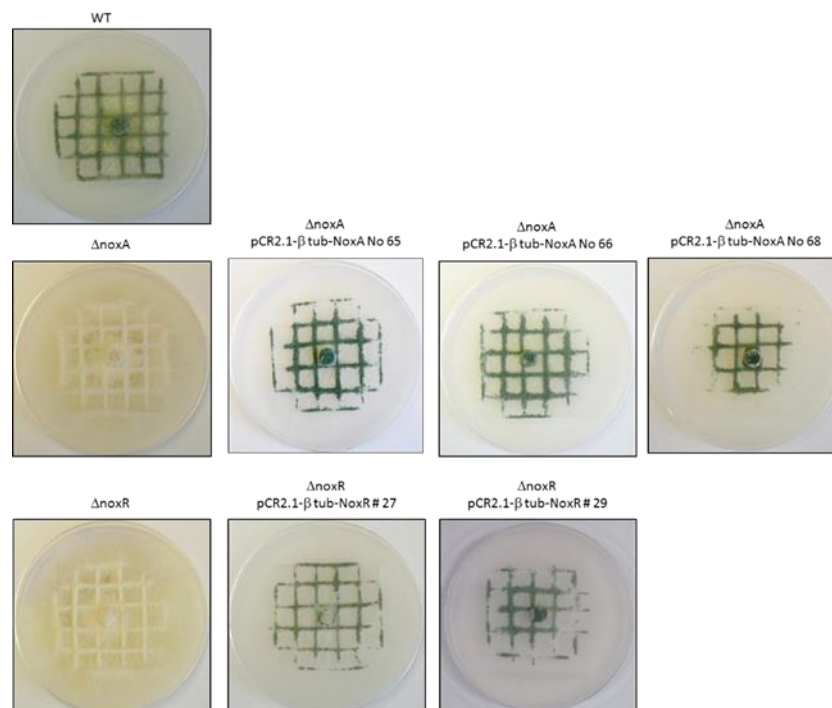
1.  $\Delta$ nox1
2. WT
3.  $\Delta$ nox1 pCR2.1- $\beta$ tub-Nox1 # 65
4.  $\Delta$ nox1 pCR2.1- $\beta$ tub-Nox1 # 66
5.  $\Delta$ nox1 pCR2.1- $\beta$ tub-Nox1 # 68
6.  $\Delta$ noxR
7. WT
8.  $\Delta$ noxR pCR2.1- $\beta$ tub-NoxR # 27
9.  $\Delta$ noxR pCR2.1- $\beta$ tub-NoxR # 29
10.  $\Delta$ nox2
11. WT
12.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 1
13.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 2
14.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 3
15.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 5

B) PCR for detecting hygromycin resistance cassette

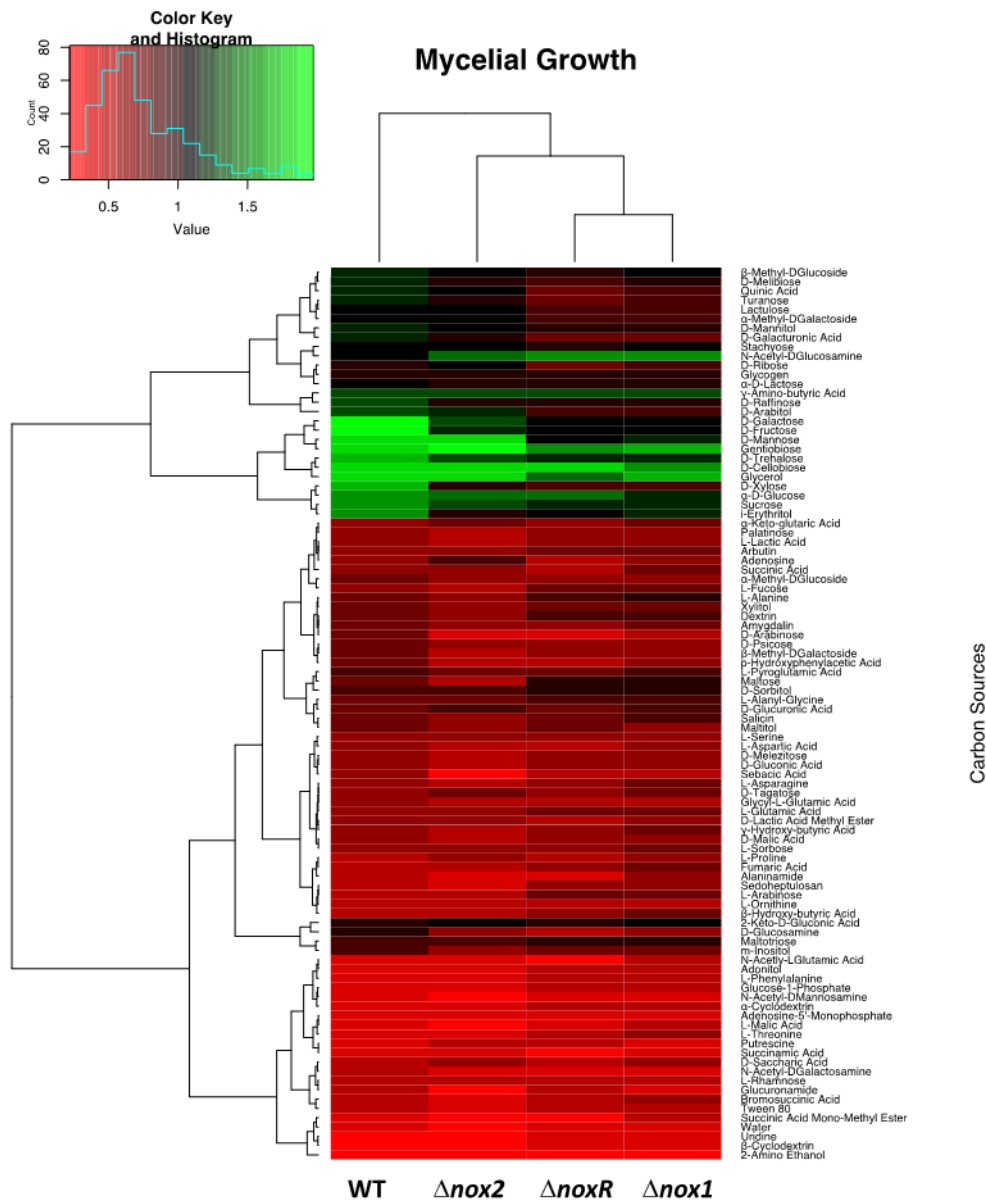


1.  $\Delta$ nox1
2.  $\Delta$ nox1 pCR2.1- $\beta$ tub-Nox1 # 65
3.  $\Delta$ nox1 pCR2.1- $\beta$ tub-Nox1 # 66
4.  $\Delta$ nox1 pCR2.1- $\beta$ tub-Nox1 # 68
5.  $\Delta$ noxR
6.  $\Delta$ noxR pCR2.1- $\beta$ tub-NoxR # 27
7.  $\Delta$ noxR pCR2.1- $\beta$ tub-NoxR # 29
8. WT
9.  $\Delta$ nox2
10.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 1
11.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 2
12.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 3
13.  $\Delta$ nox2 pCR2.1 pCR2.1- $\beta$ tub-Nox2 # 5
14. WT

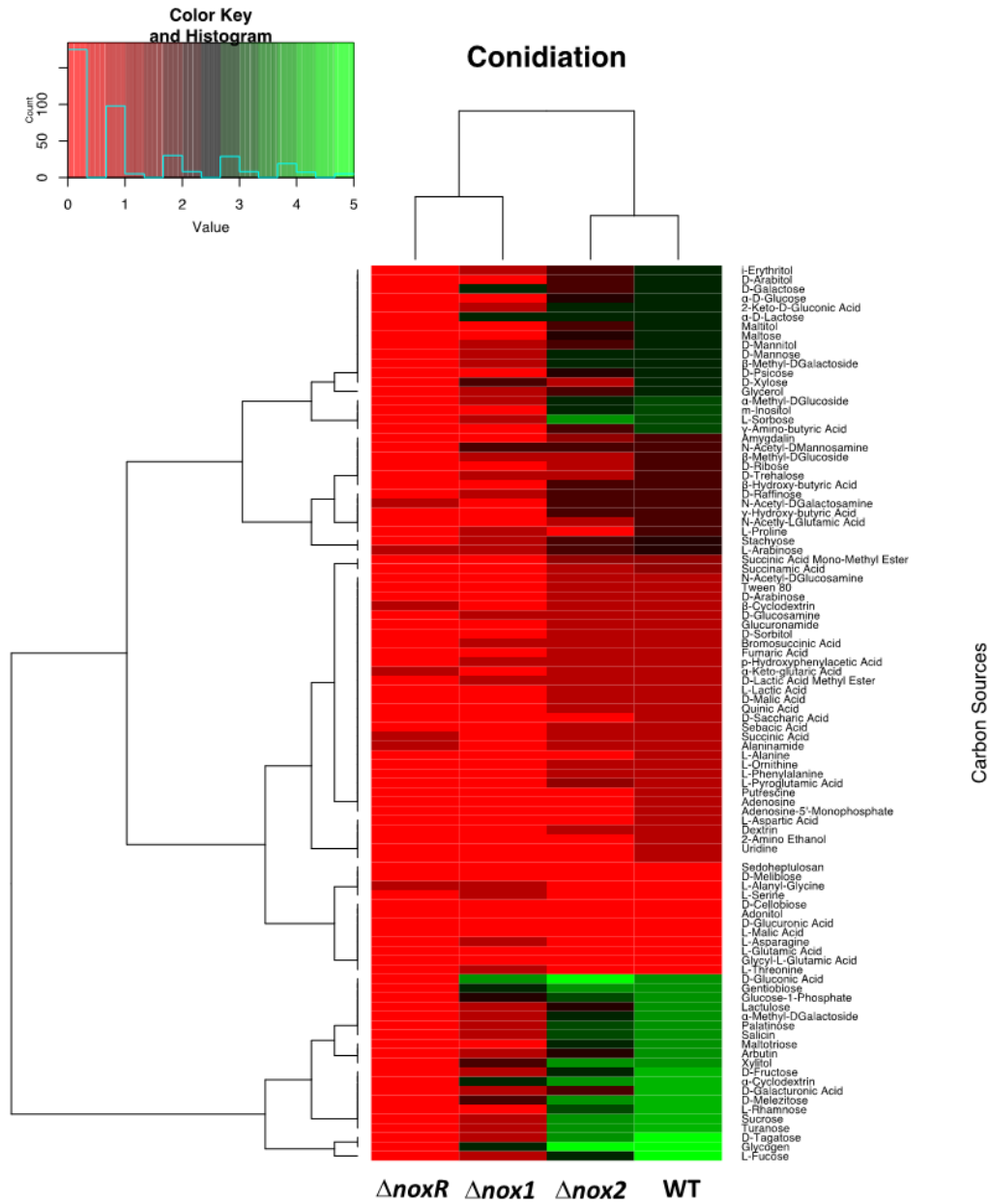
B



**Supplementary Figure S2A.** Comparative carbon sources utilization profiles of the  $\Delta nox1$ ,  $\Delta nox2$ ,  $\Delta noxR$  mutants and *wt* of *T. atroviride*. The cluster analyses are shown as colored mosaic attached to dendrograms. (A) Results of growth were assessed after 72 h of incubation at 25 °C, based on OD<sub>750</sub>. Biolog Phenotype Microarray System™ was used, with the four strains grown at 95 carbon sources using FF Plates.



**Supplementary Figure S2B.** Comparative conidiation of WT,  $\Delta nox1$ ,  $\Delta noxR$  and  $\Delta nox2$  strains after 5 d of grown on 95 carbon sources using FF Plates from Biolog Phenotype Microarray System™. The color code indicated from red (no conidiation) to pale green (abundant conidiation)



**Supplementary Figure S3.** Roles of CO<sub>2</sub> and *Trichoderma* VOCs in plant growth promotion. The effect of CO<sub>2</sub> was decreased/suppressed by addition of 100 mM of Ba(OH)<sub>2</sub> (bottom left), leaving the system under the VOCs action. The effect of *Trichoderma* VOCs was decreased/suppressed by addition of activated charcoal (bottom right), leaving the system under CO<sub>2</sub> action.

