INVESTIGATION OF THE INTERACTION BETWEEN α-PINENE AND ETHYLENE IN THE LEAVES OF TOMATO PLANTS

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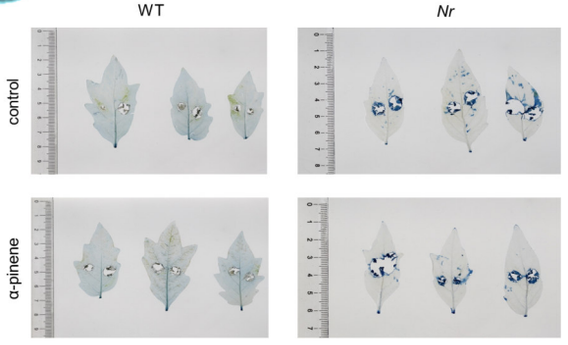
INTRODUCTION

Volatile organic compounds (VOCs) play a major part in internal and between plant **communication** constitutively and in cases of environmental stresses whether arise abiotic or biotic stress factors (Rosenkranz et al., 2021). VOCs emitted by plants also have a significant role in communication with members of unrelated plant species, which can lead to **altered defence**, growth and reproductive patterns in the reciever plant (Brosset et al. 2021), eventually causing community level **adaptation**. Although it should be mentioned that on **cellular level** or at larger scale, these effects are mainly concentration-dependent to support the variety of messages and maybe to evit other species eavesdropping. **Monoterpenes** e.g. α-pinene, are important constituents of the VOC blend in plant-plant communication and **internal signalling** as well, however the precise details of it's impact on intracellular reactions still remains largely unclear (Singh et al.). VOCs also acts as defence keys during pathogen or herbivore attacks and induce **multilevel immune responses**, meanwhile altering phytohormone induced pathways. The relation between salicylic acid and α-pinene is well proven (Riedlmeier et al.), just as the interaction between salicylic acid (SA) - jasmonic acid (JA) – ethylene (ET) in regulating defence mechanisms (Aerts et al. 2020), however, if there is a direct link between **ethylene and α-pinene** is not yet investigated.

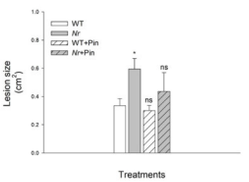
This study tends to elucidate the role of α-pinene and ethylene interaction in the defence mechanisms of **tomato plant** (*Solanum lycopersicum L.*), inoculated with necrotrophic pathogen, ***Botrytis cinerea***. Apart from defining the optimal period of α-pinene pretreatment our goal was to improve defence mechanisms against *Botrytis c.* infection.

RESULTS



***Botrytis cinerea* infected tomato leaves**

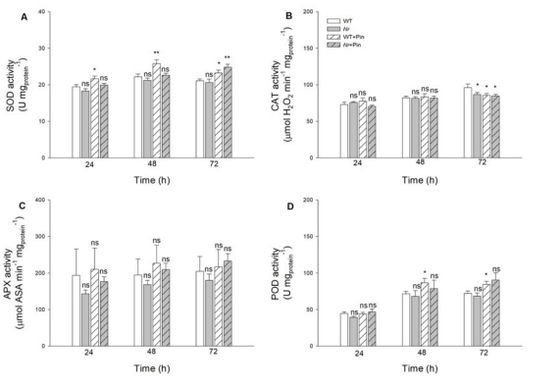
1. Evans blue dye
2. Lesion size



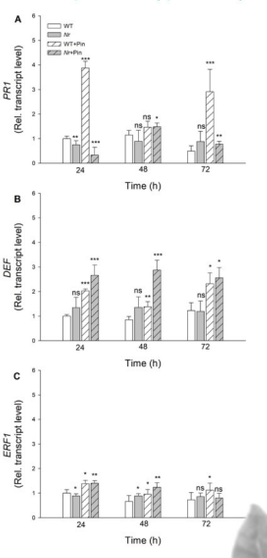
MATERIALS AND METHODS

- *Solanum lyopersicum* L. wild-type (WT, Alisa Craig) and ET receptor mutant *Never ripe* (Nr) plants were treated in the experiments.
- Pinene treatment: 0,6 µmol/ 2,6 ppm α-pinene dissolved in hexane
- Superoxide and hydrogen-peroxide productions were determined spectrophotometrically as described by Iqbal et al. (2022).
- The enzymatic activities of SOD, CAT, and POD antioxidants were detected using a spectrophotometer as illustrated by Horváth et al. (2015).
- GST enzyme activity was measured spectrophotometrically (Gallé et al. 2009).
- The detection of the expressions of key phytohormone marker genes were performed by qRT-PCR using a method reported by Czékus et al. (2020).
- To assess viability by the state of the cell membranes, we measured malondialdehyde content (Horváth et al. 2015).
- Electrolite leakage was measured (EL) (Sun et al. 2010).
- Apoptotic lesions were detected with Evans blue dye (Tóth et al. 2020).
- Data presented are average values from at least three independent experiments. Statistical analysis were performed using T-probe (mean+SE; n=3).

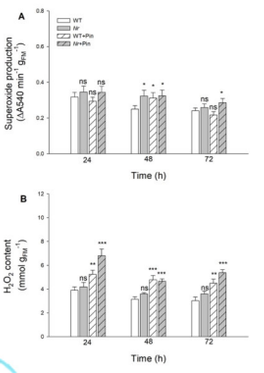
Antioxidant enzyme activity



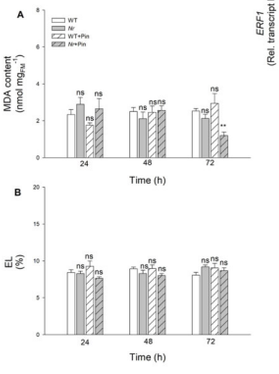
Gene expression (qRT-PCR)



Reactive oxygen species' levels



Cell vitality



CONCLUSIONS

- A milder imporvement in defence against *Botrytis cinerea* could be observed as a decrease in lesion size after a-pinene pretreatment.
- SA marker gene (PR1) showed significantly higher expression levels in WT plants, reflecting that α-pinene induced defence must be ethylene dependent.
- JA (*DEF*) and ethylene (*ERF1*) marker gene levels rose significantly, but this effect did not seem ethylene dependent.
- After treatment, significantly more H_2O_2 were produced , meanwhile $O_2^{\cdot-}$ and NO production were left almost unaffected.
- The activity of superoxide dismutase enzyme increased in the leaves of both genotypes, although it was lower in *Nr* leaves, guaiacol peroxidase showed similar activity.
- Catalase activity and ascorbate peroxidase changed to a much lesser extent in the leaves of WT plants, whereas it increased more significantly in the *Nr* leaves.
- Based on our results a 48 h α-pinene pretreatment has the most beneficial effects on defence responses, however not all of our experiments point out to the same optimum.

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