

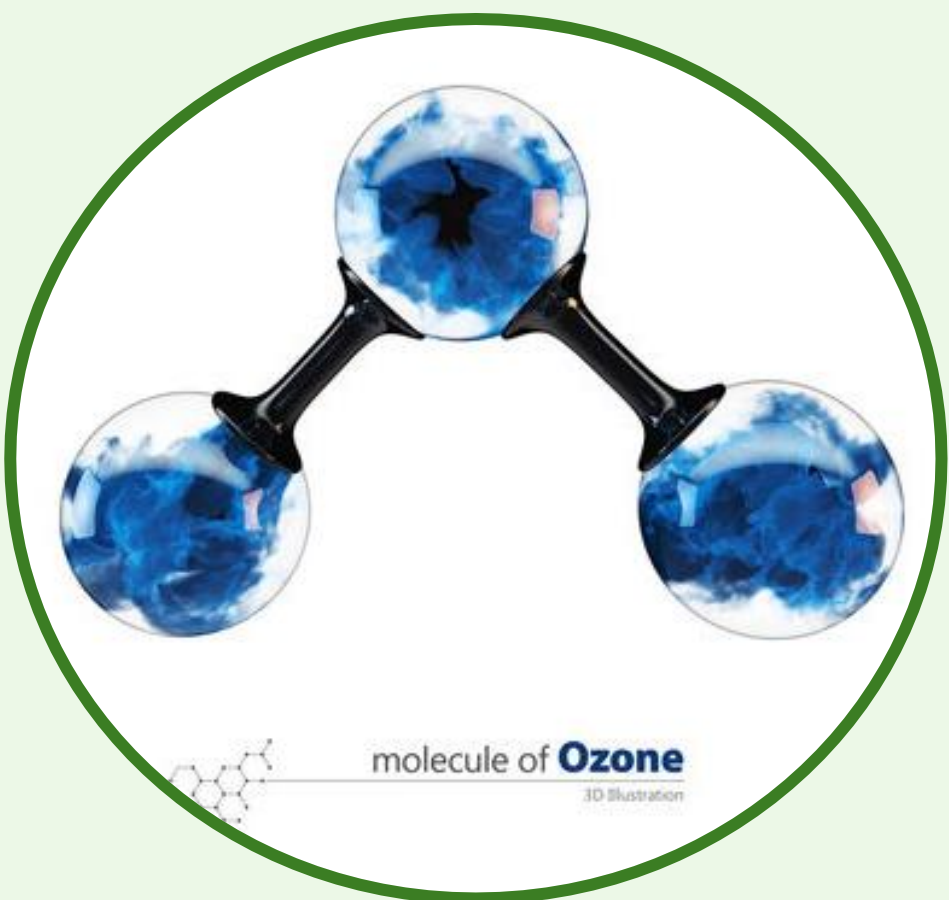
Ozone and plant defenses: a new strategy to protect plant health?

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Introduction

In a world increasingly threatened by climate change, the agricultural sector faces several challenges to sustain the rising demand for food while minimizing its environmental impacts. With growing awareness on the harmful effects of traditional pesticides and fertilizers, the development of innovative and eco-friendly strategies to protect plant health has become pivotal for sustainable agriculture. In this scenario, ozone (O₃), a powerful oxidizing agent, presents a promising eco-friendly alternative due to its rapid degradation and the absence of harmful residues in the environment. However, despite its potential, the molecular mechanisms underlying O₃ role in plant defenses are not fully understood.



Aim

Uncover the biological mechanisms triggered by O₃ on plant growth, development and defense

Materials and methods

Open-field experiment

Plants were grown in pots containing sandy soil and were treated twice or trice a week with tap water ozonated for 30 min (T1) or 1h (T2).



Hydroponic experiment

Lettuce plants were grown in 4.5L of Hoagland nutrient solution under nutritional stress (20% nutrient solution). O₃ was applied directly to the nutrient solution for 15 or 30 min.



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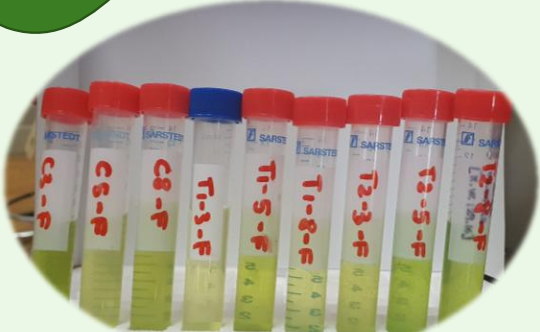


28 days

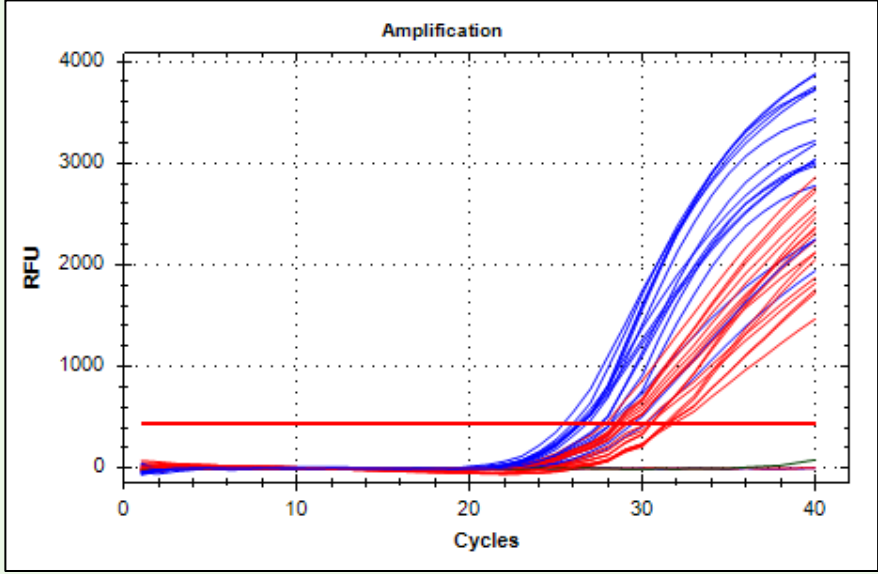
Evaluation of leaf and root dry weight and chlorophyll content.



Chl extraction



The expression of defense-related genes, such as *PAL*, *CHS*, *PR1* and auxin-responsive genes, such as *ARF1*, have been analyzed to unveil the pathways triggered in plants by O₃.

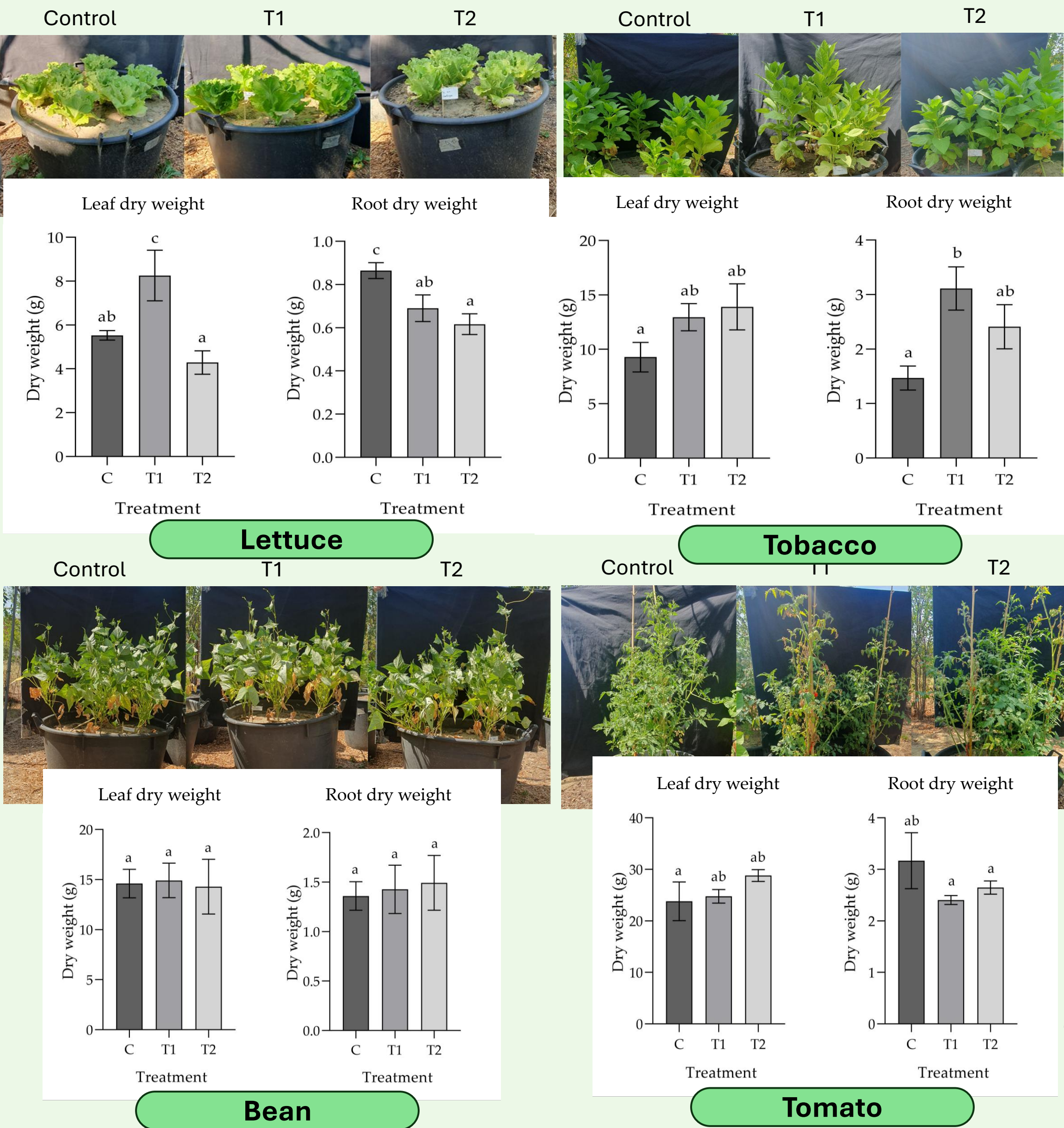


Conclusions

Ozonated water was shown to have a significant impact on plant, growth development and defenses. However, its effects were highly depended on plant species, O₃ concentration and application method. Nevertheless, in all the experiments O₃ was shown to stimulate plant defenses mainly through the induction of PR proteins, and in the specific case the induction of *PR1* which could suggest the activation of the SA signaling pathway.

Further studies need to be carried out to completely understand the molecular mechanisms involved in ozone activity, especially focusing on the more complex plant-pathogen system. All these information will be useful to support the efficacy of O₃ in crop protection.

Results and discussion



The application of water ozonated with the T1 treatment determined a statistically significant increase in lettuce leaf dry weight, while a trending decrease was observed after the T2 treatment. On the contrary both treatments caused a significant reduction in root dry weight. In tobacco, both ozonated water treatments determined an increase in leaf and root dry weight, with a significant increase in root dry weight after the T1 treatment. Concerning both tomato and bean no statistically significant increase in biomass were observed with both ozonated water treatments.

Figure 1 – Dry weight of root and leaves of plants treated with ozonated water in the open filed experiment.

In lettuce and bean the T2 treatment determined a significant upregulation of *PR1*, suggesting the activation of the salicylic acid (SA) pathway. In bean, the *PAL* gene was also upregulated after the T2 treatment potentially leading to an increase in secondary metabolites. In tobacco and tomato an increase in *PR1* expression was also observed after the T1 treatment.

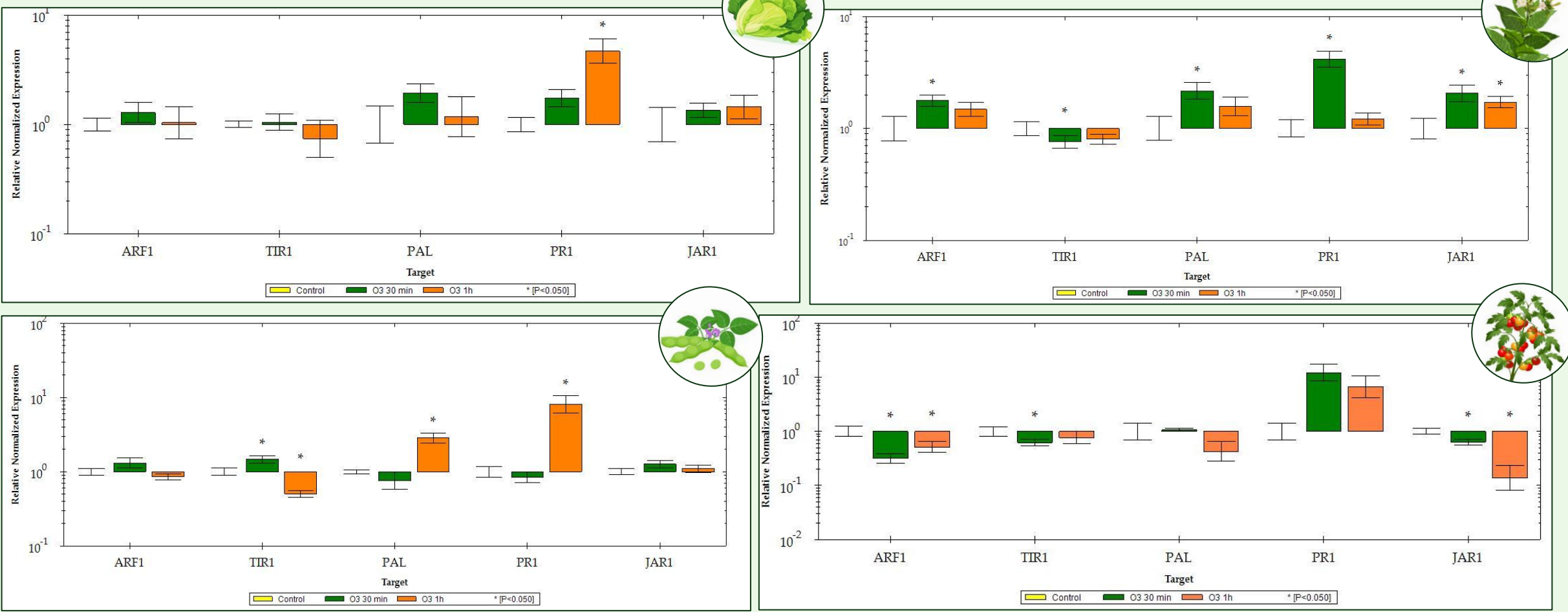


Figure 2 – Gene expression analysis of defense-related genes in potted plants irrigated with ozonated water (30 min, T1 treatment; 1 h, T2 treatment) and leaves of plants treated with ozonated water in the open filed experiment.

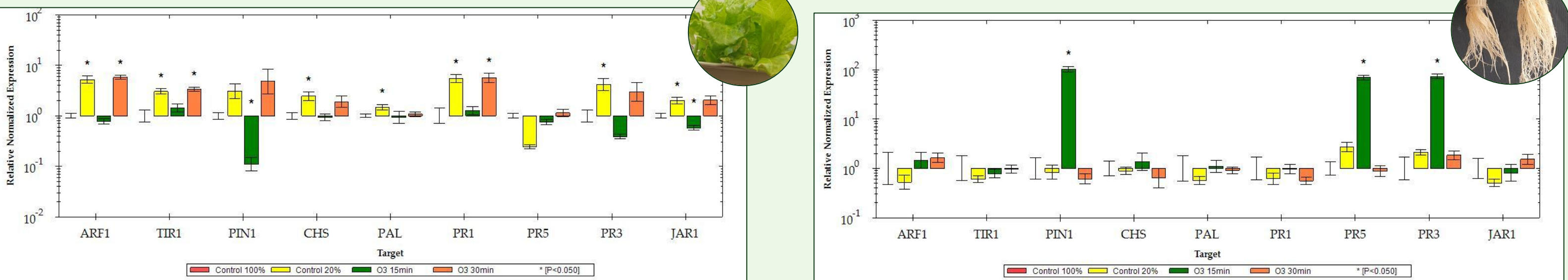


Figure 3 – Gene expression analysis of defense-related genes of leaves (A) and roots (B) of *L. sativa* hydroponically grown plants under nutritional stress treated with O₃ for 15 min (T1) and 30 min

Results showed an opposite expression profile between leaf and roots. In particular, the treatment with O₃ for 30 mins determined a significant upregulation in the genes involved in the auxin pathway and of *PR1*. On the contrary, in roots the treatment with ozone for 15 mins determined the upregulation of *PR5* and *PR3* suggesting the activation of both the salicylic and jasmonic acid pathways. Moreover, the *PIN1* was also upregulated which could result in an increased efflux of auxin.

Acknowledgments

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