Ph.D. Project Proposal

Doctoral Program in Evolutionary Biology and Ecology

Title: **Biofortification of edible plants with zinc to counteract contamination by pathogenic enterobacteria**

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Introduction:

Around the world, people eat fresh fruits and vegetables, which are vital components of a balanced diet. Although their consumption is undeniably associated with great benefits for human health, it is important to consider that a significant portion of food poisoning outbreaks is attributable to minimally processed fruits and vegetables. Initially, it was thought that this was caused by post- harvest contamination of the surface due to poor sanification practices, but more recent researches have shown that pathogenic enterobacteria can invade and multiply inside plant tissue. Studies have, in fact, provided evidence that *Salmonella enterica*, which is primarily transmitted through contaminated food, has the ability to invade, multiply, and persist within the tissues of plants, thereby transforming plants into potential reservoirs of this pathogenic bacterium (Schikora et al., 2011). This underscores the urgent need to understand the underlying mechanisms that modulate the interaction between plant hosts and human pathogens.

When encountering phytopathogens, plants initiate a response by activating defense mechanisms that depend on intricate signalling pathways. This activation leads to transcriptional reprogramming, oxidative burst, and hormonal changes within the plant, collectively working to counteract the pathogen's attack (Birkenbihl et al., 2017). Recent evidence suggests that one defence mechanism employed by plants is the intoxication of bacteria with metals, in particular zinc (Visconti et al., 2022).

This project proposal aims to characterize the molecular aspects of the plant-pathogen interaction through an experimental infection model of *Arabidopsis thaliana* (AT) and *Salmonella Typhimurium* (STM). By investigating the complex interplay between zinc defence signalling pathways and the immune response of AT to STM, this study will elucidate the contribution of zinc in augmenting plant resistance against pathogens and will significantly contribute to the prevention of STM contamination in fresh food.

Methodologies:

This project involves various experimental approaches, including inoculation studies, growth inhibition assays, gene expression analysis, and nutritional analysis. Experiments aim to assess the impact of zinc biofortification on reducing the colonization, survival, and growth of STM in different edible plant species. Furthermore, the research aims to unravel the underlying molecular mechanisms involved in zinc-induced resistance, including the activation of plant defence responses and potential alterations in nutritional composition.

The investigation into the biofortification of edible plants with zinc to counteract STM contamination can be enhanced by employing methodologies that offer a more comprehensive understanding of the mechanisms and effectiveness of zinc biofortification.

1. Metabolomics Analysis: Metabolomics is a cutting-edge technique that allows for the comprehensive analysis of small molecule metabolites present in plants. By using advanced analytical instruments such as mass spectrometry and nuclear magnetic resonance, metabolomics can provide a detailed understanding of the metabolic changes induced by zinc biofortification. This approach can identify specific metabolites or metabolic pathways that are influenced by zinc and potentially contribute to enhanced resistance against STM.

2. High-Throughput Sequencing: Technologies such as RNA sequencing, can provide a comprehensive analysis of the transcriptome of biofortified plants. By comparing the gene expression profiles of biofortified and non-fortified plants, this approach can identify key

genes and regulatory pathways that are activated or suppressed by zinc biofortification. It can uncover novel genes involved in plant defense mechanisms against STM and provide insights into the molecular basis of zinc-mediated resistance.

3. Imaging Techniques: Advanced imaging techniques, such as confocal microscopy and electron microscopy, can provide detailed visualization of the interactions between biofortified plants and STM. These techniques allow for the examination of the spatial distribution of bacteria within plant tissues and provide insights into the impact of zinc biofortification on bacterial colonization and penetration.

By incorporating these advanced methodologies into the study of biofortification with zinc, we can gain a more comprehensive understanding of the molecular, metabolic, and microbial aspects involved in enhancing plant resistance against STM. These approaches offer new insights into the mechanisms underlying zinc-mediated protection and contribute to the development of effective strategies to mitigate contamination risks.

Objectives and Expected Results:

Objective: To investigate the impact of zinc biofortification on the plant immune response and the expression of defense-related genes in the presence of STM.

Expected results: The study is expected to reveal upregulated defense-related genes and enhanced plant immune responses in biofortified plants, indicating the potential of zinc biofortification in boosting plant resistance against STM contamination.

Reference:

1. Birkenbihl R. P., Liu S., Somssich I. E. (2017). Transcriptional events defining plant immune responses. Curr. Opin. Plant Biol. 38, 1–9. doi: 10.1016/J.PBI.2017.04.004.
2. Schikora A, Virlogeux-Payant I, Bueso E, Garcia AV, Nilau T, et al. (2011) Conservation of Salmonella Infection Mechanisms in Plants and Animals. PLoSONE 6(9): e24112. doi:10.1371/journal.pone.0024112.
3. Visconti S, Astolfi ML, Battistoni A, Ammendola S. (2022). Impairment of the Zn/Cd detoxification systems affects the ability of Salmonella to colonize Arabidopsis thaliana. doi:10.3389/fmicb.2022.975725.