Fatima Naiyyira

naiyyirafatima373@gmail.com

"Initial Raindrops Activate Predictive Immunity in Plants Before Pathogen-Favorable Wet Conditions Develop"

Abstract
There are a number of varying environmental stimuli to which plants are exposed that influence development, productivity and immune responses in them. Mechanical signals (such as those generated by raindrops impacting leaf surfaces) serve as warning cues for plants to activate their immune defenses. Therefore, in this study, we've developed our hypothesis on whether the way in which the mechanical signals produced by the first rainfall drop landing on a leaf will result in the early activation of an immune response. By elucidating this mechanism, this study aims to better understand the plant innate immunity and environmental signal integration.

Introduction
Plants can perceive and respond to environmental stimuli, ensuring survival under varying biotic and abiotic stresses. For instance, vibrations produced by buzzing bees trigger release of pollens from poricidal anthers(Vallejo-Marín, 2021) and plants exposed to traditional music like mantras have shown increased amylase activity and chlorophyll levels during seed germination(Godhani et al., 2025). Also, when plants are subjected to wet conditions they can create an immune response to pathogens(Yang et al., 2021). While we know about the use of chemical and molecular signals in plants that mediate immunity to pathogens, the role of mechanical stimuli, particularly those caused by raindrops on dry leaves, as early indicators for immune activation remains underexplored. This research will provide insight into how plants interact with their environment and will influence the development of new agricultural techniques for improving crop resilience.

Literature Review

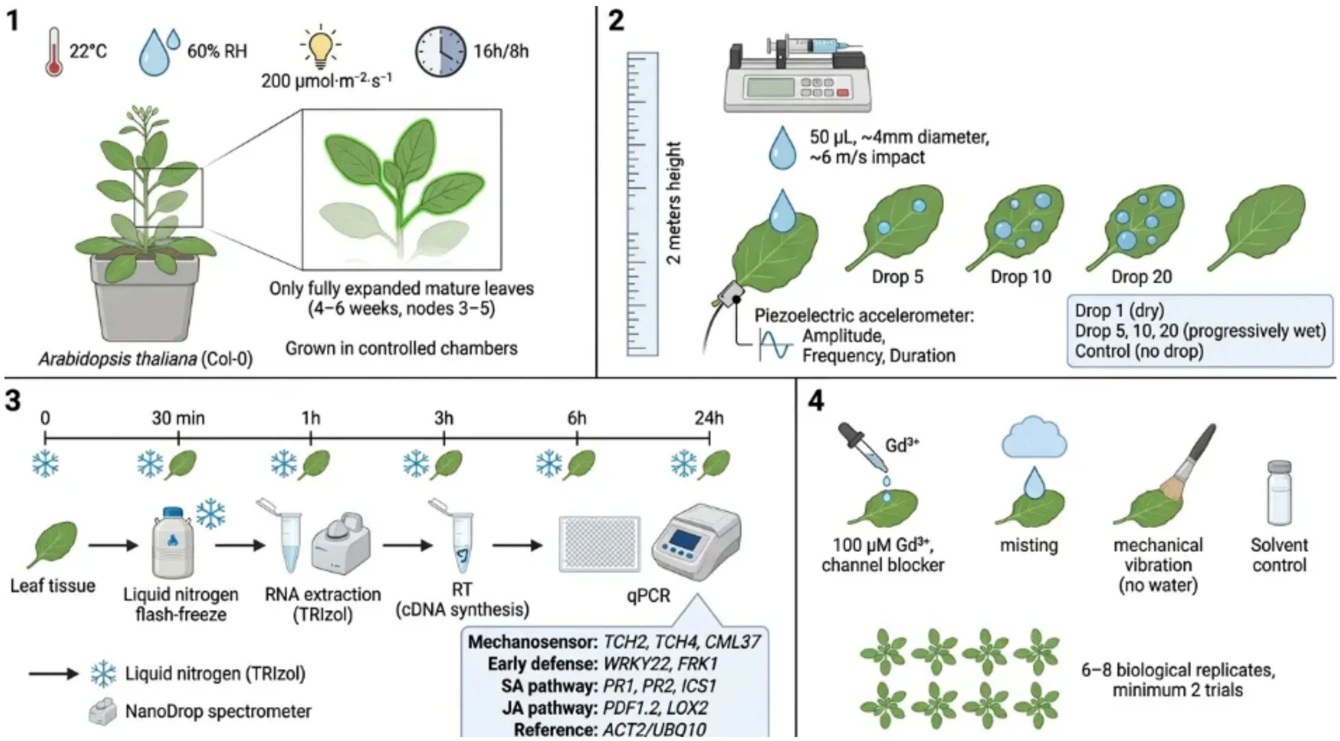
Mechanosensitive channels and receptors are present in the plants and respond to physical stimuli like touch, wind, and vibrations, converting them into biological reactions. Studies have shown that mechanical touch may result in modified gene expression and systemic resistance(Darwish et al., 2022). Mechanical signals like rain drops falling onto the leaves function as warnings for the plants to initiate their immunity system due to the reason that rain serves as the medium of dispersal of pathogen and propagules(Wu et al., 2024). Rainfall exerts distinct mechanical stimuli on leaf surfaces. On dry leaves, raindrop impact is sensed as a mechanical stimuli by trichomes which are hair like structures present on the epidermis. As a response, trichomes trigger calcium waves and activate defense genes before infection can take hold. When trichomes are mechanically stimulated, intercellular calcium waves are concentrically propagated away from them, MAPKs are activated, and the calcium- and calmodulin-binding 2 transcription activator (CAMTA)-regulated immune response is initiated(Darwish et al., 2022; Matsumura et al., 2022). Whereas, on pre-wetted leaves, a water film is formed that dampens raindrop impact energy which weakens early immune activation(Gao et al., 2025). Additionally, plant immune systems are known to prime defenses in response to environmental cues before pathogen attack.(Hönig et al., 2023) Primed plants show enhanced expression of SA and JA pathway genes, leading to significantly reduced disease susceptibility

Methodology

Arabidopsis thaliana (Col-0) plants, with well-characterized immune response, will be grown in controlled chambers at 22°C, 60% relative humidity, 200 μmol m^{-2} s^{-1} light intensity under 16h/8h photoperiod.Only fully expanded mature leaves (4-6 weeks old) from the 3rd to 5th nodal position will be selected to ensure developmental uniformity.(Cai et al., 2024) Plants will be well-watered from below to maintain dry leaf surfaces for at least 24 hours before treatment. Raindrops will be simulated using a programmable syringe pump delivering 50 μL drops ,having ~4mm diameter, from 2m height, achieving ~6m/s impact velocity comparable to natural rainfall.(Gao et al., 2025) Treatments will include single drops applied to completely dry leaves (Drop 1), drops applied after progressive surface wetting (Drop 5, Drop 10, Drop 20), and a no-drop control. A miniature piezoelectric accelerometer attached to the petiole base will record vibration amplitude, frequency spectrum, and duration for each impact position. (Gao et al., 2025)Leaf tissue will be flash-frozen in liquid nitrogen at 0, 30 minutes, 1, 3, 6, and 24 hours post-treatment. RNA will be extracted using TRIzol reagent, quality-verified by NanoDrop (A260/A280 ≥ 1.8), and converted to cDNA using SuperScript III reverse transcriptase.(qPCR) will quantify expression of mechanosensitive genes (*TCH2*, *TCH4*, *CML37*), early defense markers (*WRKY22*, *FRK1*), salicylic acid pathway genes (*PR1*, *PR2*, *ICS1*), and jasmonic acid pathway genes (*PDF1.2*, *LOX2*), normalized against reference genes *ACT2* and *UBQ10* using the 2^(-ΔΔCt) method. To test the priming window, a single early raindrop will be applied to dry leaves.This will be followed by pathogen inoculation (*Pseudomonas syringae* pv. tomato DC3000, 10^6 CFU/mL) at 0, 2, 6, 24, and 48 hours post-raindrop. Disease severity will be assessed at Days 3, 5, and 7 using a 0-5 severity index, lesion diameter measurements, and bacterial growth quantification by serial dilution plating and pathogen-specific qPCR. A parallel plant cohort receiving identical raindrop treatment but no pathogen will be sampled at the same timepoints for defense gene expression analysis. To confirm mechanical signal specificity, mechanosensitive channels will be blocked using 100 μM gadolinium chloride (Gd^{3+}) applied 1 hour before raindrop treatment. Additional controls will include water-only misting (no mechanical impact), mechanical vibration without water (soft brush stimulation), and solvent controls. All experiments will include 6-8 biological replicates per treatment, conducted across at least two independent trials. Data will be analyzed using two-way ANOVA with Tukey HSD post-hoc testing, Pearson correlation between vibration parameters and gene expression, and Kruskal-Wallis test for non-normally distributed disease severity data. Benjamini-Hochberg false discovery rate correction will be applied for multiple gene comparisons. All pathogen work will be conducted under BSL-1 containment following institutional biosafety protocols.

Conclusion

The first drops of rain hitting dry leaves create mechanical signals that activate plant immune responses prematurely. This mechanoperception "primes" the plant defense gene expression and enhances the pathogen resistance of the plant to Pseudomonas syringae. The inhibition of mechanosensitive channels confirms the specificity of the mechanotransduction pathway. These findings represent an important connection between environmental mechanical signals and immune signaling.



References

- Cai, J., Panda, S., Kazachkova, Y. *et al.* A NAC triad modulates plant immunity by negatively regulating *N*-hydroxy pipecolic acid biosynthesis. *Nat Commun* **15**, 7212 (2024).
- Darwish, E., Ghosh, R., Ontiveros-Cisneros, A., Tran, H. C., Petersson, M., De Milde, L., Broda, M., Goossens, A., Van Moerkercke, A., Khan, K., & Van Aken, O. (2022). Touch signaling and thigmomorphogenesis are regulated by complementary CAMTA3- and JA-dependent pathways. *Science Advances*.
- Gao, Z., Lin, J., Ma, J., Wei, H., Dong, X., & Qiu, B. (2025). Experimental investigation of droplet impact behavior considering leaf curvature and vibration effects. *BMC Plant Biology*.
- Godhani, N., Jani, G., & Gadani, M. (2025). A biochemical evaluation of starch, amylase & chlorophyll under the influence of mantra exposure in germinating wheat. *International Journal of Bioscience and Biochemistry*.

- Hönig, M., Roeber, V. M., Schmülling, T., & Cortleven, A. (2023). Chemical priming of plant defense responses to pathogen attacks. *Frontiers in Plant Science*.
- Matsumura, M., Nomoto, M., Itaya, T., Aratani, Y., Iwamoto, M., Matsuura, T., Hayashi, Y., Mori, T., Skelly, M., Yamamoto, Y. Y., Kinoshita, T., Mori, I. C., Suzuki, T., Betsuyaku, S., Spoel, S. H., Toyota, M., & Tada, Y. (2022). Mechanosensory trichome cells evoke a mechanical stimuli-induced immune response in *Arabidopsis thaliana*. *Nature Communications*.
- Nevard, L., Russell, A. L., Foord, K., & Vallejo-Marín, M. (2021). Transmission of bee-like vibrations in buzz-pollinated plants with different stamen architectures. *Scientific Reports*.
- Zixuan Wu *et al.* Coherent spore dispersion via drop-leaf interaction.*Sci. Adv.***10**,eadj8092(2024)
- Yang, S., Cai, W., Shen, L., Wu, R., Cao, J., Tang, W. et al. (2022). Solanaceous plants switch to cytokinin-mediated immunity against Ralstonia solanacearum under high temperature and high humidity. Plant, Cell & Environment, 45, 459–478.