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Microplasma Stimuli for Efficient Molecular Introduction and Physiological Activation in Plants Cell

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1. Introduction

Plant cells are enclosed by a rigid cell wall, making the direct introduction of macromolecules such as proteins more challenging compared to animal cells. However, we have previously demonstrated successful genome editing by introducing a Cas9/sgRNA complex into *Nicotiana tabacum* callus cells using a microplasma technique [1]. Additionally, we successfully introduced and expressed a GUS plasmid. In this study, we report on the mechanisms by which plasma facilitates molecular delivery into plant cells, with a particular focus on the roles of reactive oxygen species (ROS) and electrical stimulation.

2. Experimental setup

Callus cells derived from *Nicotiana tabacum* were used as target samples. FITC-dextran (250 kDa; Sigma-Aldrich) was used as the introduced molecule. The callus was placed in a 3.5 cm dish on a grounded electrode, and a discharge electrode was positioned 1 mm above the callus surface. Plasma treatment was performed using a sinusoidal voltage of 11 kVpp for 20 ms, applied twice. After plasma exposure, 5 μL of FITC-dextran solution was dropped onto the treated area, followed by incubation for 60 minutes. The samples were then washed and observed under a fluorescence microscope.

To assess the role of ROS in molecular introduction, we tested the following inhibitors: catalase (CAT) to scavenge extracellular H_2O_2, N-acetyl-L-cysteine (NAC) to suppress overall intracellular and extracellular ROS, and diphenyleneiodonium (DPI) to inhibit NADPH oxidase (RBOH) activity.

3. Results and Discussion

Plasma-treated cells exhibited approximately 2.8-fold higher fluorescence intensity compared to the untreated control, indicating enhanced molecular uptake. However, the introduction efficiency significantly decreased in all inhibitor-treated groups (CAT, NAC, DPI), as shown in Figure 1. These findings suggest that ROS play a crucial role in plasma-enhanced molecular introduction. In particular, the CAT result indicates the involvement of extracellular H_2O_2, while the strong inhibitory effect of DPI highlights the importance of NADPH oxidase-mediated ROS generation.

NADPH oxidase transports electrons across the plasma membrane to generate superoxide (O_2^-) on the apoplastic (extracellular) side. Due to its instability, O_2^- is believed to rapidly convert into H_2O_2, which can diffuse through the membrane and influence intracellular signaling or membrane structure. This may lead to the activation of endocytosis. Figure 2 shows a schematic diagram of the mechanism of molecular introduction by plasma stimulation. Plasma-induced molecular delivery requires not only the formation of physical entry routes through the cell wall but also ROS-mediated chemical stimulation. Future studies including real-time visualization of membrane dynamics and selective inhibition of endocytic pathways will be essential to elucidate the detailed mechanism.

4. Conclusion

This study demonstrates that successful molecular delivery into plant cells using microplasma requires both physical disruption of the cell wall and the induction of endocytosis via a combination of plasma-induced electrical stimulation and NADPH oxidase–mediated ROS generation. Notably, H_2O_2 generated near the plasma membrane plays a critical role in triggering endocytosis, and NADPH oxidase activity is a key factor in this process.

Acknowledgments

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References

[1] Y. Ikeda et al., Japanese Journal of Applied Physics, 62, SL1015 (2023).

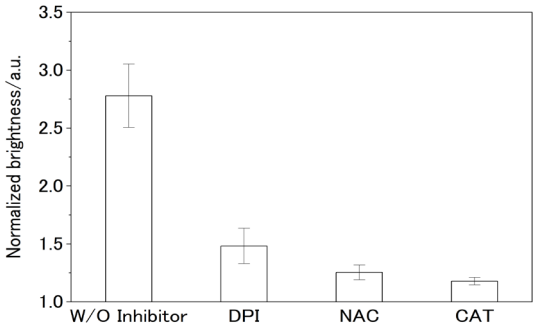

Figure 1. Effect of ROS-related inhibitors on plasma-induced enhancement of molecular introduction.

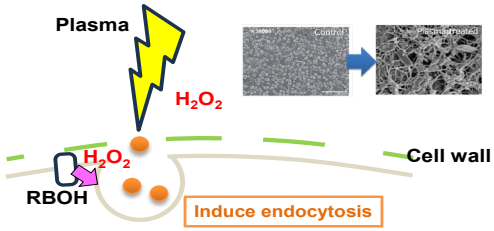

Figure 2. Schematic diagram of the mechanism of molecular introduction by plasma stimulation.