Molecular introduction into pollen by surface discharge treatment

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Abstract: We treated kiwifruit pollen with surface discharge and introduced FITC-Dextran, which has a molecular weight like Cas9, into pollen with high introduction efficiency. Green fluorescence was also observed around the nucleus, confirming that the molecules were introduced into sperm cells. These results suggest that surface discharge treatment could enable the direct acquisition of genome-edited individuals without the need for tissue culture.

1. Introduction

The pollen of angiosperms contains the plant's reproductive cells, namely sperm cells, and serves to transport them to the egg cell. Introducing CRISPR/Cas9 into pollen makes it possible to directly edit the genome that will be passed on to the next generation, such as sperm cells or fertilized eggs. Furthermore, pollinating with this pollen allows for the direct acquisition of genome-edited plants without needing tissue culture. In this study, we report the successful introduction of a fluorescent molecule (FITC-Dextran) with a molecular weight of 250 kDa, similar to CRISPR/Cas9, into kiwifruit pollen using surface discharge treatment.

2. Methods

Fig. 1 shows a schematic diagram of the surface discharge treatment method. FITC-Dextran was used as the introduced substance. After dropping 100 µL of a solution containing FITC-Dextran at a concentration of 5 µg/µL in 5% PBS into a 2 cm dish, kiwifruit pollen was added. The electrode (Ni) for surface discharge treatment was set 1 mm above the liquid surface. A sinusoidal high voltage was applied to generate surface discharge. The discharge time was set to 20 ms. After surface discharge treatment, the pollen was left to stand in the dark for 1 hour. Then, by adding trypan blue solution, the fluorescence of FITC-Dextran in the PBS solution outside the pollen was quenched, and the pollen was observed under a fluorescence microscope.

3. Results and Discussion

Fig. 2 shows fluorescence observation images of pollen after surface discharge treatment. The entire germinated pollen, including the pollen tube and the area around the nucleus, shows green fluorescence, indicating the successful introduction of FITC-Dextran by surface discharge treatment. We have previously demonstrated that gaps are formed in the cell walls of plant cells treated with microplasma, but unlike normal plant cells, pollen cell walls have a two-layer structure with an inner and outer wall, making it more difficult to create gaps, but gaps were also formed in the cell walls of pollen treated with surface discharge. FITC-Dextran reaches the cell membrane through this gap. FITC-Dextran that reaches the cell membrane is introduced into the pollen by endocytosis in the same way as in micro-plasma-treated plant and animal cells.

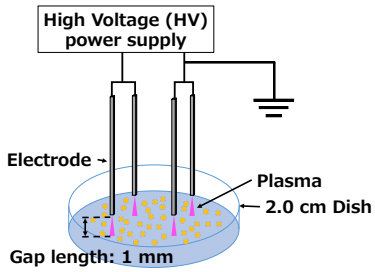

Fig. 1. Experimental schematic diagram of the surface discharge treatment method.

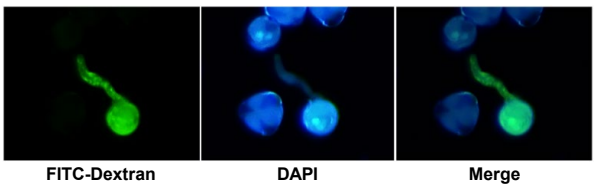

Fig. 2. Fluorescence observation of kiwifruit pollen after molecular introduction by surface discharge treatment.

4. Conclusion

We successfully introduced FITC-Dextran, which has a molecular weight similar to CRISPR/Cas9, into kiwifruit pollen using surface discharge treatment. Green fluorescence was observed around the nucleus, indicating that the molecules were introduced into the sperm cells. These results suggest that surface discharge treatment could enable the direct acquisition of genome-edited individuals without the need for tissue culture.

Acknowledgement

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References

[1] Y. Ikeda et al., Jpn. J. Appl. Phys., 62, SL1015 (2023).