Effects of Reactive Oxygen Species on Plasma Molecular Introduction into Plant Cells by Plasma Treatment

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Abstract: In this study, we investigated the effects of ROS on molecular introduction into plant cells by plasma treatment. Callus was treated with NAC, a reactive oxygen scavenger, before plasma treatment to confirm the effect of chemical factors. Callus with ROS suppressed by NAC treatment showed almost no molecular introduction compared to callus without NAC treatment. These results suggest that electrical stimulation and ROS of plasma production induce endocytosis in both plant and animal cells.

Keywords: Plant cell, Molecular introduction, ROS

1. Introduction

We are investigating techniques to introduce genes and molecules into animal cells by plasma treatment [1-3]. We have shown that stimulation of the plasma membrane activates a transport mechanism of the cell membrane called endocytosis, by which molecules are introduced into the cell. We are also working to apply this technology to plant cells. The technology to easily and efficiently introduce proteins directly into plant cells for applications such as breeding and flowering control through genome editing is expected to be established but does not currently exist. Introducing proteins directly into plant cells is more difficult than in animal cells because cell walls surround the cells. However, the authors succeeded in genome editing by introducing a complex of Cas9 and sgRNA into tobacco callus culture using the plasma method. In our previous studies, electrical stimulation of plasma production contributes to the introduction of molecules into plant cells. However, the effect of chemical factors is not apparent. In this study, we investigated the effects of ROS on molecular introduction into plant cells by plasma treatment.

2. Experimental setup

A schematic diagram of the experiment is shown in Fig. 1. Tobacco callus (*Nicotiana tabacum*) was used as the target. The callus was placed in a 3.5 cm dish. Plasma treatment was performed at a distance of 1 mm from the tip of the electrode to the callus. The applied voltage was a sinusoidal 11 kVpp. After plasma treatment, 10 µl of FITC-dextran (FD250S: Sigma Aldrich) solution (10 µg/µl) was dropped into the cells, allowed to stand for 30 minutes, washed, and the treated callus was observed under a fluorescence microscope (MVX10: Olympus). Callus was treated with N-Acetyl-L-cysteine (017-05131: Wako) solution (10 mM) for 15 minutes, and an active oxygen scavenger before plasma treatment was also treated to confirm the effect of the chemical factor.

3. Results and discussion

The fluorescence microscopy results are shown in Fig. 2. Plasma-treated callus fluoresced green. NAC-treated callus

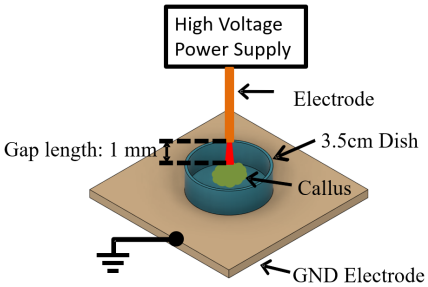
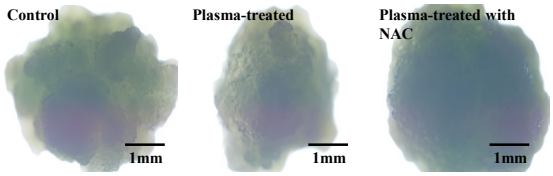
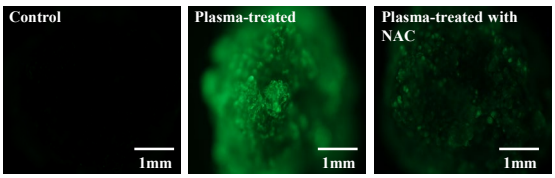


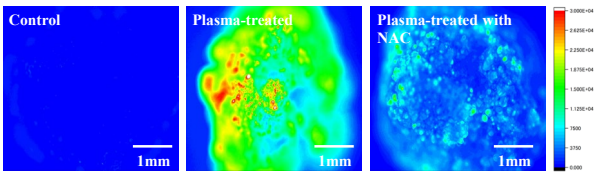
Fig. 1. Schematic of a plasma treatment system.



(a) Bright-field image



(b) Dark-field fluorescence image



(c) color-mapped image

Fig. 2. Fluorescence microscopy results.
Left: Control, Center: Plasma treated, Right: Plasma treated with NAC.

had weaker fluorescence intensity than a plasma-treated callus. A comparison of the average fluorescence intensity of the callus is shown in Fig. 3. The average luminance was calculated by dividing the sum of the luminances of each pixel in the image of the callus area identified in the darkfield fluorescence image by the total number of pixels in the callus area. It should be noted that the number of pixels at the callus/background boundary was counted higher than it actually was for this result due to the blurred focus at the callus/background boundary. These calculations were performed using analysis software (Origin Lab Pro: Lightstone).

As shown in Fig. 2, the brightness of the callus in which ROS was suppressed by NAC treatment was lower than that of the callus in the normal treatment in which ROS was not suppressed. These results confirm the contribution of reactive oxygen species to the mechanism of molecular introduction into plant cells by plasma treatment. Our previous study reported ROS inhibition with NAC reduced gene transfection efficiency into animal cells in plasma treatment [3]. We also reported that ROS and electrical stimulation of plasma generation might contribute to the induction of endocytosis in animal cells [2]. These results suggest that electrical stimulation and ROS of plasma production induce endocytosis in both plant and animal cells. The OH radicals have also been reported to decompose cell wall pectin [4]. Fig. 3 shows the results of SEM observation of the cell wall surface of the plasma treatment and control. The cell wall surface of plasma-treated callus shows the formation of pores that provide a pathway for molecules to reach the plasma membrane. Therefore, the mechanism of molecular introduction into plant cells by plasma treatment is thought to be that molecules that reach the cell membrane through the introduction pathway created in the cell wall are introduced into the cell by endocytosis. SEM observation of the cell wall surface after plasma treatment of NAC-treated callus is necessary to confirm the effect of ROS on cell wall decomposition and removal. SEM observations of the cell wall surface after plasma treatment of ROS-suppressed callus will be reported on the day of the presentation.

4. Conclusion

In this study, we investigated the effects of ROS on molecular introduction into plant cells by plasma treatment. Callus was treated with NAC, a reactive oxygen scavenger, before plasma treatment to confirm the effect of chemical factors. Callus with ROS suppressed by NAC treatment showed almost no molecular introduction compared to callus without NAC treatment.

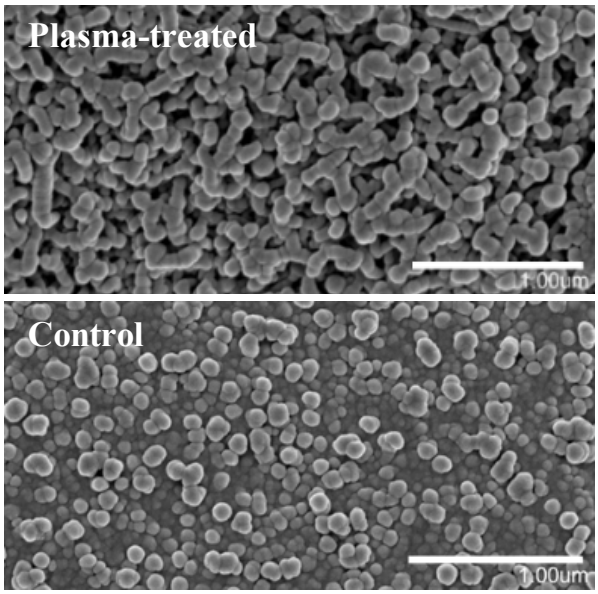


Fig. 3. SEM observation of the cell wall surface of tobacco callus.
(Upper: Plasma-treated callus, Lower: Control.)

Acknowledgments

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5. References

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