Introduction mechanism of fluorescent molecules into tobacco cells in plasma gene / molecules introduction method

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Abstract: In this study, the introduction mechanism of the fluorescent molecules into the tobacco leaf is investigated. By using inhibitor of clathrin-mediated endocytosis, the contribution of endocytosis in the introduction mechanism was observed. As a result of SEM observation of the leaf surface after the plasma irradiation, the cuticula layer on the epidermal cells were removed. Clathrin-mediated endocytosis and removal of the cuticula layer by plasma made it possible to introduce large molecules of 2 MDa into tobacco epidermal cell.

Keywords: Plant cell, Tobacco, Endocytosis, Cuticula

1. Introduction

Medical, agricultural, and fishery field require a versatile technique of gene transfection. Conventional techniques have their own problems such as cytotoxic, low introduction efficiency, or low versatility. As a solution to those problems, we have been studied new gene transfection method into animal cells by using micro plasma [1-4]. Protein introduction into plant cells by plasma jet has already been reported [5]. However, problems remain such as low introduction efficiency, introduction stability, no introduction of large molecules.

In this study, we investigated the introduction of the large fluorescent molecules up to 2 MDa into the tobacco leaf by atmospheric discharge plasma. We also investigated the effect of endocytosis inhibition on molecular introduction to elucidate the introduction mechanism.

2. Experimental setup

Schematic of plasma treatment system is shown in Fig. 1. The tobacco (*Nicotiana benthamiana*) leaf was used as target plant cell. After plasma irradiation, the 8μl TE-buffer solution including 2 MDa fluorescent molecule that is FITC-Dextran (Sigma-Aldrich: FD2000S) was dropped. The sinusoidal voltage is applied for 13 ms. The frequency is 20 kHz. The discharge current is 0.5 App or 1 App. The tobacco leaf was placed on a grounded conductive gel pad (Nippon Medical Next: Thermoguard 51-7810). The discharge gap length was 1 mm from the needle electrode (Ni: φ 140 μm) to the surface of the leaf. After plasma irradiation, the FITC solution was dropped immediately and was left for an hour. An hour later, tobacco leaf was washed by water and observed by fluorescent microscope. In addition, to elucidate the introduction mechanism, Tyrphostin A23(TCI: T3503) was used as an inhibitor of clathrin-mediated endocytosis. Tyrphostin A23 was added dropwise on the surface of leaf 1 hour before. After 1 hour, the Tyrphostin A23 was wiped off and plasma treatment was carried out.

3. Results and discussion

Fig. 2(a) shows the fluorescent image of tobacco leaf after plasma irradiation without inhibitor. Many green fluorescent cells were observed near the plasma irradiation area. These cells show puzzle piece like shape are epidermal cells. Though epidermal cell is covered by cuticula, fluorescent molecule is introduced into cell. This suggests that a pathway which through cuticula is created by plasma.

Fig. 2(b) shows the fluorescent image with Tyrphostin A23 inhibitor. When an inhibitor was used, introduction was hardly observed. The area ratio of the fluorescent part was 1/10 or less. This result shows that clathrin-mediated endocytosis is dominant in the molecular introduction mechanism of tobacco epidermal cells.

Fig. 3 shows the SEM(JEOL: JSM-7500F) observation of tobacco leaf surface. In Fig. 3(a), there are puzzle piece like layer on the surface of leaf. These layers consist of cuticula and extra-cuticula. As shown in Fig. 3(b), the cuticula layer on surface of the plasma irradiated epidermal cell is thin and flat. The cuticula layer becomes thinner or is removed by plasma irradiation. This makes the fluorescent molecule accessible to the epidermal cell. In addition, clathrin-mediated endocytosis was induced by plasma irradiation, and the fluorescent molecules were introduced into the cells. Fig. 4 shows the schematic diagram of the mechanism of introducing gene and molecules into plant cells by plasma irradiation.

4. Conclusion

As long as authors know, there is no report on the success in introduction of such a large molecule into a plant cell by discharge plasma. The fluorescent molecule was accessible to the epidermal cells because the cuticula layer was removed by plasma irradiation. The fluorescent molecules were introduced into epidermal cells by clathrin-mediated endocytosis. The large molecule is introduced into pant cell by plasma generated by discharge in the air. The irradiation time is as short as 13 ms, there is no temperature rise at the irradiated part.

Since fluorescent molecules with a molecular weight of 2 MDa were introduced, it is expected that this method introduces genes of the same molecular weight.

5. References

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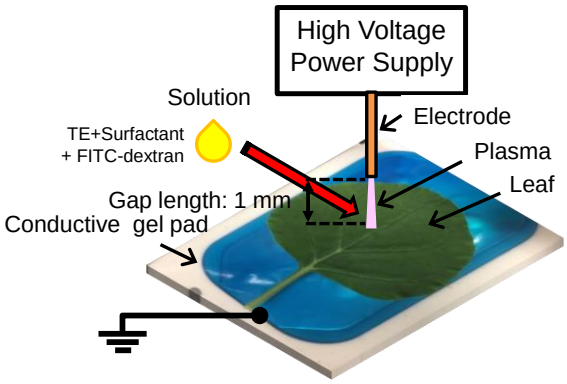
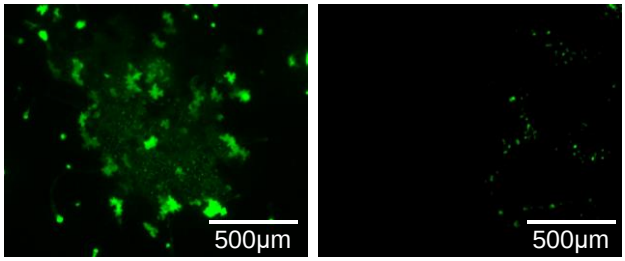
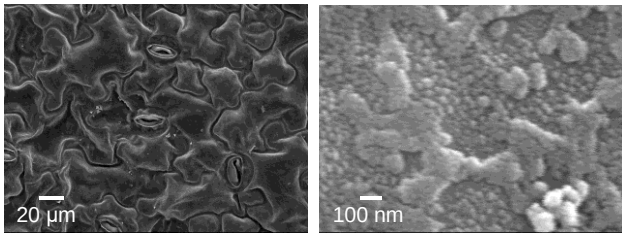


Fig. 1. Schematic of plasma treatment system.
The sinusoidal voltage is applied. The frequency is 20 kHz. The discharge current is 0.5 App or 1.0 App. The tobacco leaf was placed on a grounded conductive gel pad.

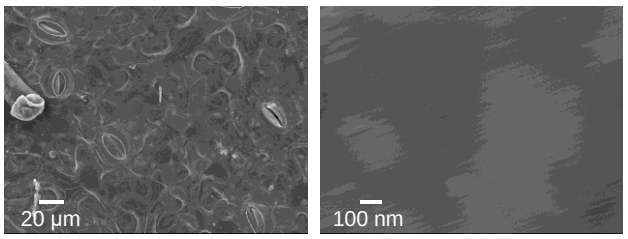


(a) Without inhibitor (b) Tyrphostin A23

Fig. 2. Fluorescent images after plasma treatment.
The frequency is 20 kHz. The discharge current is 0.5 App. Irradiation time is 13 ms.



(a) Control



(b) Plasma irradiation

Fig. 3. SEM observation of Tobacco leaf surface.
The discharge current is 0.7 App. Irradiation time is 25 ms. The SEM observation sample was prepared 24 hours after plasma irradiation.

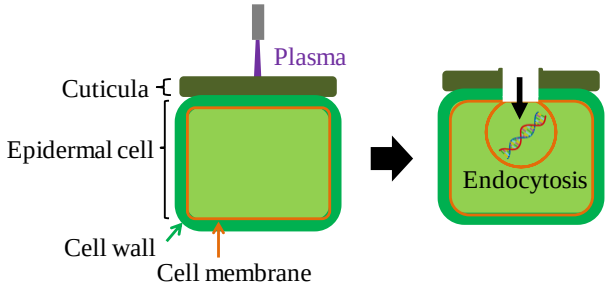


Fig. 5. Schematic diagram of the mechanism of introducing gene and molecules into plant cells by plasma irradiation.