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Establishment of experimental systems to analyze the effects of low-temperature plasma on plant growth and the initial intracellular responses using *Marchantia polymorpha*

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Atmospheric-pressure cold plasma irradiation has been reported to promote seed germination, plant growth, enhance immunity, and more. For the practical application of plasma technology in agriculture, it is essential to understand the effects of plasma-generated factors on plants at the cellular and molecular levels. To address this, we explored the use of *Marchantia polymorpha* as a model organism for plasma plant science.

Currently, *M. polymorpha* is a representative model species in plant physiological research due to several advantages: its whole genome has been fully sequenced, it shares high genetic similarity with other land plants, allows easy genetic manipulation, numerous useful experimental techniques have already been developed and established, and has high regenerative ability and rapid growth. In particular, gemmae, which are clonal tissues developed from *M. polymorpha* thalli, offer a uniform genetic background and can be easily and abundantly obtained under cultured conditions. Therefore, we used gemmae in our plasma studies.

First, to investigate the long-term effects of plasma irradiation on plant growth, we established an experimental system using scalable dielectric barrier discharge (SDBD) plasma to irradiate *M. polymorpha* gemmae. As a result, SDBD plasma promoted gemmaling growth at low doses irradiation, whereas high doses inhibited growth [1]. These findings suggest that *M. polymorpha* gemmae are suitable for plasma plant studies. Additionally, we quantified the absolute levels of various plasma-generated factors that reached the gemmae in this system, including electric fields, light, and long-lived reactive species [1].

Second, to investigate initial cellular responses as short-term effects of plasma irradiation, we developed a pen-shaped dielectric barrier discharge (PDBD) plasma device capable of irradiating plant samples under a fluorescence microscope. Using gemmalings expressing GCaMP6f and HyPer7 in the cytosol—fluorescent indicators for Ca^{2+} and H_2O_2, respectively—we observed transient increases in their fluorescence levels immediately after PDBD irradiation [2]. The immediate increase in cytosolic Ca^{2+} levels upon PDBD irradiation was suppressed by lanthanum ions (La^{3+}), an inhibitor of Ca^{2+}-permeable channels in the plasma membrane, suggesting that the Ca^{2+} influx into the cytosol occurred from the extracellular space through these channels. In contrast, using diphenyleneiodonium (DPI), an inhibitor of the reactive oxygen species (ROS)-producing enzyme NADPH oxidase/respiratory burst oxidase homolog (RBOH), did not prevent the increase in cytosolic H_2O_2, indicating that H_2O_2 generated by the PDBD device was directly introduced into the cells [2]. Currently, we are conducting engineering analyses to characterize the factors generated by the PDBD device.

By integrating engineering-based analyses of plasma sources with genetic and cell biological approaches, we aim to elucidate the molecular mechanisms underlying the effects of cold plasma irradiation on plants and to identify the key contributing factors from both the plasma source and the plant side involved in these responses. We expect that the fundamental scientific findings from this study will serve as a valuable contribution to the advancement of plasma agriculture.

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