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Plasma-synthesized N_2O_5 exposure induces a systemic defense response in *Arabidopsis thaliana*

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Abstract

Dinitrogen pentoxide (N_2O_5), a unique oxidizing and nitrating compound, is a promising chemical for a variety of applications, but requires multiple dangerous raw materials (careful handling) to synthesize N_2O_5 by conventional methods. Therefore, it has not yet been used for bio-applications, especially in agriculture. Recently, we have developed a new air atmospheric-pressure plasma (APP) device/method that allows highly selective production of N_2O_5 exclusively from only air and electricity sources and that enables to expose plants to N_2O_5. To explore the applicability of APP-generated N_2O_5 in plant immunity enhancement, we investigated the immune responses (calcium response and mRNA expression) in *Arabidopsis thaliana*. The APP-generated N_2O_5 exposure induced calcium response propagation from an exposed leaf to a whole body within 10 sec and gene expression related to signaling of jasmonic acid (JA), a plant defense hormone, at 10 min. These results indicate that the APP-generated N_2O_5 can provoke plant defense response.

1. Introduction

Atmospheric-pressure plasma (APP) technology, enabling to convert air molecules into multi-functional reactive species [*e.g.*, reactive oxygen and nitrogen species (RONS)] with electricity, is of great interest and has been extensively investigated. For example, ozone (0_3) produced by the APP technology has multifunctional abilities such as disinfection and deodorizing, and has already been into practical use[1]. Recently, we have developed a new air APP device/method that allows highly selective production of dinitrogen pentoxide (N_2O_5) exclusively from only air and electricity sources [2]. N_2O_5, a unique oxidizing and nitrating compound, is a promising chemical for a variety of applications, but requires multiple dangerous raw materials (careful handling) to synthesize N_2O_5 by conventional methods. Therefore, it has not yet been used for bio-applications. Our APP device/method for N_2O_5 production does not require careful handling, complicated manufacturing equipment, and toxic substances, and have the potential to be used in scientific and industrial applications. Here, we investigated the applicability of APP-generated N_2O_5 in agriculture.

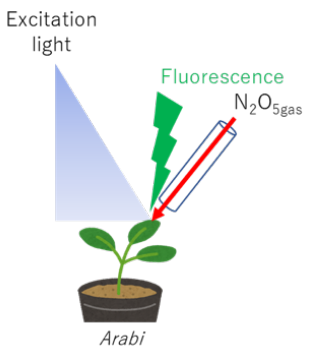

Fig. 1. Plasma-synthesized N_2O_5 exposure to *A. thaliana*

2. Experiment setup

The experiment was conducted by observing the change in the cytosolic calcium ion concentration ($[Ca^{2+}]_{cyt}$) in *Arabidopsis*

thaliana , which expresses a GCaMP3 fluorescent protein-based cytosolic Ca^{2+} sensor. Then the shoot part of the plant was collected and tested for plant defense hormone related genes expression by real time RT-qPCR. Figure 1 shows the experimental schematic diagram of the exposure plant to N_2O_{5gas}. When plant is somehow affected by N_2O_{5gas} exposure and produce a stress signal, it also produces fluorescence, which changes corresponds to a change in the $[Ca^{2+}]_{cyt}$. This fluorescence can be observed by fluorescence microscopy. After 10 min of N_2O_{5gas} exposure, the plant defense hormone jasmonic acid (JA)-related genes expression was measured by real time RT-qPCR.

3. Results and discussion

As shown in Figure 2, APP-generated N_2O_5 exposure induced the $[Ca^{2+}]_{cyt}$ signal increase within 1 min. Furthermore, local APP-generated N_2O_5 exposure to a single leaf induced an increase in $[Ca^{2+}]_{cyt}$ that spreads not only to exposed leaf but also not exposed leaves. This response is very similar to wound-induced $[Ca^{2+}]_{cyt}$ signaling, which elicits a systemic defense response by the plant defense hormone JA[3]. To verify the similarity of the reactions, the expression of JA-related genes was monitored in shoot part of plant exposed to APP-generated N_2O_5. RT-qPCR results showed that expression of JA-related genes was significantly induced compared to Air exposure (Figure 3). These results indicate that the APP-generated N_2O_5 can provoke plant systemic defense response. If it is certain that a systemic defense response is induced by APP-generated N_2O_5, which is made from ubiquitous sources (air and electricity), we can utilize APP-generated N_2O_5 as a newly pesticide instead of chemical pesticides. Furthermore, it might be an important element of sustainable agriculture.

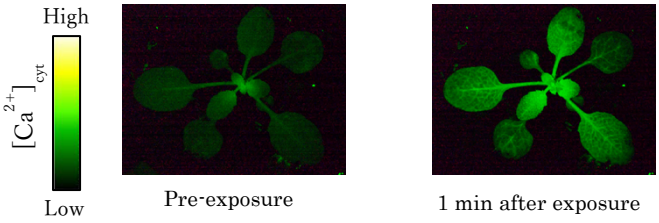

Fig. 2. Plasma-synthesized N_2O_5 exposure induces Ca^{2+} response

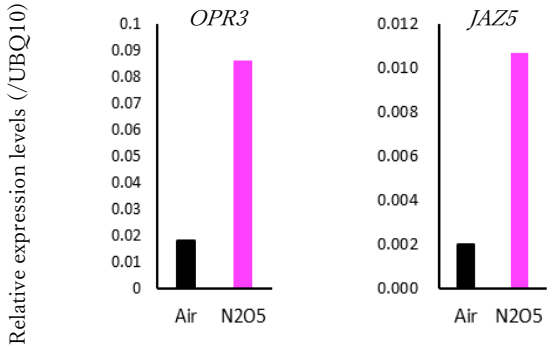

Fig. 3. Plasma-synthesized N_2O_5 exposure induces expression of JA-related genes

References

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