Nitrogen Fertilization with Plasma Generated Dinitrogen Pentoxide

S. Takeshi[1], K. Takashima[1], S. Sasaki[1], A. Higashitani[2], T. Kaneko[1]

[1] *Graduate School of Engineering, Tohoku University, Sendai, Japan.*
[2] *Graduate School of Life Sciences, Tohoku University, Sendai, Japan.*

Abstract: Recently, a method for selectively synthesizing dinitrogen pentoxide from air using discharge plasma has been established, and plasma nitrogen fixation may be useful as a nitrogen fertilizer. In this study, plasma-generated dinitrogen pentoxide was supplied to the model legume *Lotus japonicus*, and the effect of plasma nitrogen fixation was evaluated. The results showed that dinitrogen pentoxide became an essential nitrogen source for the growth of *Lotus japonicus*, depending on the method of supply.

Keywords: Nitrogen Fertilization Effect, Growth Promotion, Dinitrogen Pentoxide

1. Introduction

Currently, nitrogen fertilizers, one of the macronutrients essential for crop production, are synthesized mainly by the Haber-Bosch process, in which ammonia is synthesized from nitrogen from the atmosphere and hydrogen from fossil fuels under high temperature and pressure. Nitric acid and other nitrogenous fertilizers are also synthesized from this process. The Haber-Bosch process alone consumes fossil fuels, approximately 3-5% of the world's total production of natural gas and 1-2% of total energy production [1], and further costs for their global transportation are unavoidable.

Plasma nitrogen fixation [2], recently suggested to synthesize nitric oxides and ammonia, has advantages in the abundant resources and less requirements on the energy sources, allowing on-site generation of nitrogen fertilizers. Recently, selective plasma synthesis of dinitrogen pentoxide (N_2O_5), anhydride of nitric acid, has been established, exclusively using air and electricity [3]. N_2O_5, one of the nitrogen oxides with the highest oxidation number (+5), has not yet used widely due to risks in its conventional synthesis methods requiring multiple dangerous raw materials (careful handling). This form of nitric oxides can easily react with liquid water to form nitrate, unlike NO and NO_2 gas, thus can be easily used as a fertilizer. Also, regarding the N_2O_5 oxidization and nitration reactivity, on-site generation of N_2O_5 from air can potentiates new applications for agriculture, such as a plant disease resistance induction [4].

This plasma nitrogen fixation converting nitrogen in air into nitric acid precursor can play a significant role in fixing nitrogen even in farming field, where symbiotic rhizobia in legumes, directly converting nitrogen in air into ammonia, performs symbiotic nitrogen fixation in nature. In this study, the growth of a model legume, *Lotus japonicus*, with the plasma generated N_2O_5 was compared to the growth with symbiotic nitrogen fixation as a measure of the plasma nitrogen fixation efficacy.

2. Experimental setup and methods

Nitrogen fertilization effect of N_2O_5 was experimentally tested by two methods: one is to supply the water solution bubbled with N_2O_5 gas to form liquid phase nitrate (indirect fertilization) and the other is to expose a model legume, *Lotus japonicus*, to N_2O_5 gas (direct fertilization). The density of N_2O_5 generated from air with our plasma device [3] was kept at 7.3×10^{15} molecule/cm^3 for all cases.

Lotus japonicus were sown on agar not containing nitrogen and grown for 4 days. Nine seedlings were transplanted to a covered pot to prevent unintended nitrogen contamination and rhizobia contaminants, filled with autoclaved vermiculite without any explicit nitrogen source then cultivated in a growth chamber at 25℃ (light 12h/dark 12h). A standard nitrogen free culture medium was supplied to the pods with the liquid reservoir underneath the vermiculite storing the rest of culture solution. The water and the fertilizer in the soil were maintained through a cotton yarn dipped in the liquid reservoir. It is important to mention that the nitrogen free culture medium without KNO_3 contains sufficient amount of potassium ion.

The direct fertilization, direct exposure to highly reactive N_2O_5 gas, can induces a damage to *Arabidopsis thaliana* [4], thus the exposure time should be regulated. A set of conditions in terms of exposure time (10, 20, 30, 60, and 90 seconds) were choosen to experimentally determine a threshold to induce any visual damage symptom as the first test. The first direct fertilization experiment was conducted once a week and repeated four-times a week. We observed differences in damage development observed in one day after the N_2O_5 gas exposure.

Based on above first test, conditions to have both the nitrogen fertilization effect and the damage to *Lotus japonicus* were chosen. The second direct fertilization experiment was conducted once a week with either 30 s or 60 s exposure time. The beginning of the direct fertilization after the transplantation was varied from one to 4 weeks in order to see the difference in damage tolerance in the growth stage of the *Lotus japonicus*.

The N_2O_5 bubbling to 150 mL solution was found to capture more than 95 % of gas phase N_2O_5 as NO_3^-. The treatment duration for the indirect fertilization was adjusted to nitrate ion concentration in a liquid fertilizer of 7.8 mM for a liquid fertilizer case. The liquid fertilizer solution, mixture of 7.8 mM of KNO_3 with the nitrogen free culture medium, were supplied to two pods after removing the nitrogen free medium in the reservoir. In the same day, N_2O_5 gas bubbled nitrogen free medium was prepared, following neutralization to pH 6.8. Also on the same day, a rhizobium strain, *Mesorhizobium loti* MAFF #303099, were inoculated to three pods, which would become a natural source of nitrogen for *Lotus japonicus*. Comparison among those cases gives an evaluation on nitrogen fertilization effect of N_2O_5. The growth was photographed and the fresh weight of each group were evaluated at 5 weeks for the indirect fertilization and at 8 weeks for the second direct fertilization.

3. Results and discussion

At 5 weeks after cultivation for indirect N_2O_5 exposure experiment, yellowed leaves, a nitrogen deficiency symptom, were clearly observed in the negative control group with nitrogen free medium, while the N_2O_5 indirect fertilization did not show the same symptom. Also, the N_2O_5 indirect fertilization group shows that *Lotus japonicus* can grow to the same extent as the cases with KNO_3 and rhizobial inoculation, thus the N_2O_5 indirect fertilization can suppress a nitrogen deficiency. It is also important to mention that root nodules, inferring infection of the rhizobial strain, were formed only in the pod with rhizobial inoculation. Therefore, one can conclude that the N_2O_5 indirect fertilization was effective to suppress the nitrogen deficiency.

Figure 1 shows the first direct fertilization result where the longer the exposure time, the more significant the discoloration as a damage symptom. In particular, leaves of *Lotus japonicus* with the exposure time than 60 seconds were almost discolored and growed slowly. Therefore, we considered 30 seconds to be the threshold for the onset of symptom. Figure 2 shows the *Lotus japonicus* damage recoverly and growth through observation one day before and after each 30-second exposure. The results indicate that the growth stage may apprarently differ the damage symptom severity, whose mechanism are under investigation.

At 7 weeks for the second direct fertilization, the yellowed leaves were barely seen for the direct fertilization cases while decoloring damage on leaves were found for most of the N_2O_5 exposed samples. The plant height were clearly higher than the negative control for 30 seconds exposure case. Therefore, regardless to the damage symptom as a side effect, the direct N_2O_5 gas exposure may have significat fertilization effect, supported with the following fresh-weight measurement. For the 60 seconds exposure case, however, the degree of the decoloring damage was severer than those for 30 seconds' cases. There was a tendency that the less plant height and sever damage were correlated with the beginning of the direct fertilization.

The fresh weight of *Lotus japonicus* samples in Figure 2 were measured at 8 weeks. The fresh weights of the direct fertilization group for 30 seconds' exposure were statistically larger than those of the negative control regardless to the beginning of the exposure start date. Therefore, it can be interpreted that the direct fertilization has the nitrogen fertilization effect. The 60-second cases show that the fresh weights with the direct fertilization started from two weeks after the cultivation is significantly less than the negative control. The latter beginning of the direct fertilization appears to improve the fresh weight and appears less damage. This is interpretable as insufficient damage tolerance at the younger stage of *Lotus japonicus* to the 60 seconds N_2O_5 exposure.

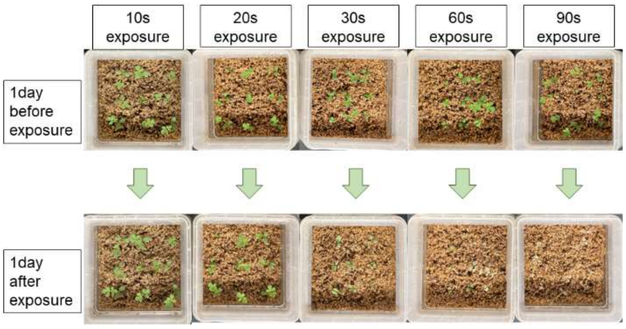

Fig. 1. Relationship between exposure time and damage symptoms at the first exposure (8 days after replanting).

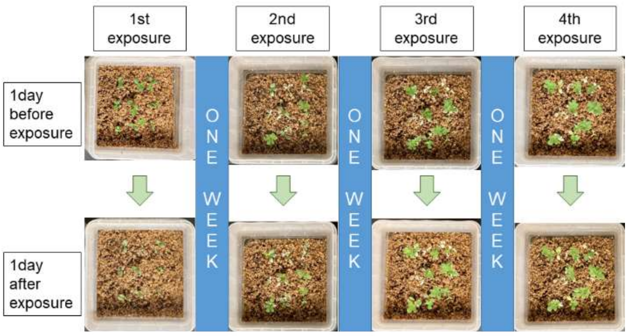

Fig. 2. Differences in discoloration damage symptoms for each 30-second exposure.

It is important to mention that at the given condition, the more growth at the earlier beginning of the nitrogen supply can be expected owing to more nitrogen supply. According to the fact that the growth with the nitrogen fertilizer KNO_3 were significantly heavier than any direct fertilization cases. These results and discussion infer that perheaps, the damage induced by the N_2O_5 gas exposure might be mitigated for well-grown plant, and then nitrogen fertilization effect can overwhelm the induced damage.

The further study to control the damage to the air-synthesized N_2O_5 direct exposure might improve the growth of *Lotus japonicus*.

4. Conclusion

The nitrogen fertilization effect of plasma-generated N_2O_5 was experimentally investigated. The indirect exposure by N_2O_5 bubbling brought negligible damage and the nitrogen fertilization effect comparable to the nitogen fertilizer and symbiotic nitrogen fixation groups at the given condition. In the direct exposure, the nitrogen fertilization effect were observed but the plant height and the fresh weight was far less than the nitrogen fertilizer, simply due to approximately 1/10 or less nitrogen supply. The amount of the nitrogen supply at the given exposure scheme was limited by the damage caused by the N_2O_5 gas exposure, clearly observed a day after the exposure. The damage symptom is significantly improved by delaying the beginning of the N_2O_5 gas exposure. Therefore, it can be suggested that the N_2O_5 gas exposure duration control along with the growth stage of the plant may allows more nitrogen supply within the growth period.

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