Note

Seed Disinfection Effect of Atmospheric Pressure Plasma and Low Pressure Plasma on *Rhizoctonia solani*

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Gas plasma generated and applied under two different systems, atmospheric pressure plasma and low pressure plasma, was used to investigate the inactivation efficacy on the seed-borne pathogenic fungus, *Rhizoctonia solani*, which had been artificially introduced to brassicaceous seeds. Treatment with atmospheric plasma for 10 min markedly reduced the *R. solani* survival rate from 100% to 3% but delayed seed germination. The low pressure plasma treatment reduced the fungal survival rate from 83% to 1.7% after 10 min and the inactivation effect was dependent on the treatment time. The seed germination rate after treatment with the low pressure plasma was not significantly different from that of untreated seeds. The air temperature around the seeds in the low pressure system was lower than that of the atmospheric system. These results suggested that gas plasma treatment under low pressure could be effective in disinfecting the seeds without damaging them.

Key words : Gas plasma / Seed disinfection / Rhizoctonia solani.

Seed-borne diseases refer to plant diseases that are transmitted by seeds. Damping-off caused by *Rhizoctonia solani* Kühn is an important seed-borne disease and can be carried by seeds of vegetables such as Japanese hornwort, and solanaceous and brassicaceous crops (Neergaard, 1977). Treatment by dressing seeds with or dipping them in fungicide is commonly used to prevent the disease. Such chemical seed treatments are very effective in disease control but in recent years there has been increasing public demand for ecofriendly alternative methods.

Hot water and hot air treatments are effective nonchemical methods for seed disinfection. Hot water treatments are generally completed in 10 - 30 minutes at 45 - 55°C, but the water temperature and treatment time must be carefully controlled to inactivate the pathogens without damaging the seeds (Babadoost, 1992). Hot air treatments do not require a drying process after treatments, but they usually take several days to inactivate the pathogens, for example, 5-7 days at 75°C for black rot (*Xanthomonas campestris* pv. *campestris*) disinfection (Shiomi, 1992).

Plasma treatment has attracted a lot of attention as a method for inactivating microorganisms, especially as a substitute method for medical instrument sterilization (Iseki et al., 2010). In plasma discharge, a source gas is dissociated into various species such as electrons, ions, atoms, and radicals which interact with and inactivate the microorganism (von Keudell et al., 2010). However, in the field of agriculture there have been few reports concerning the inactivation effect of plasma on plant pathogens (Iseki et al., 2010; Selcuk et al., 2008).

Vacuum equipment is not needed to produce atmospheric plasma, thereby reducing the complexity and cost of the system (Yu et al., 2007). We have previously reported that atmospheric gas plasma can inactivate the pathogenic fungi *Rhizoctonia solani* cultured on potato dextrose agar (PDA) (Nishioka et
This result suggested that gas plasma was effective in inactivating the seed-borne pathogen. However, the seed disinfection effect with gas plasma was not clarified. On the other hand, it is possible that the electric discharge in low gas pressure would generate a large volume of uniform plasma in comparison with discharge at atmospheric pressure, and low pressure plasma might be better suited for the future application of gas plasma to seed disinfection. Furthermore, Dhayal et al. (2006) used low pressure RF argon gas discharge, which was generated with a radio-frequency power supply, for surface modification of safflower seeds and reported that plasma treatment could increase the germination rate. In this study, we evaluated the disinfection effect of gas plasma produced under two types of systems, atmospheric pressure or low pressure, on seeds inoculated with the seed-borne pathogen, R. solani.

The effect of fungal inactivation by plasma was evaluated using brassicaceous seeds (Brassica campestris var. amplexicaulis) as a model. The seeds were obtained from Takii Co., Ltd. (Kyoto, Japan). These seeds were soaked for one hour in sterilized water and dried by blotting them with sterile paper under a laminar flow hood. For inoculation with R. solani, the seeds were transferred onto PDA plates, preincubated with the fungus (the same strain as in Nishioka et al., 2013) for 4 days at 25°C, and incubated at 30°C for about 10 h.

For atmospheric gas plasma treatment, five seed samples were set in each well of a 48-well microplate. The samples were set under plasma discharge of the previously developed atmospheric pressure plasma apparatus (Misawa et al., 2013), and the plasma was generated by AC high voltage discharge with argon. The voltage and frequency applied to electrodes were 10 kV and 10 kHz, respectively. The argon gas flow rate was 3L /min. The treatment time and the distance from the plasma source to the sample surface were 10 min and 30 mm, respectively, which were found to be the best conditions for sterilizing R. solani culture disks in our previous study (Nishioka et al., 2013). After the plasma treatment, treated seeds were placed on a new water agar plate for detection of R. solani. The new plates were incubated for 6 days at 30°C and the rate of the seeds with surviving R. solani was considered as the fungal survival rate. This treatment was repeated six times.

The fungal survival rate of the plasma-treated seeds decreased to 3% whereas before plasma treatment it was 100%. This result indicated that gas plasma could inactivate R. solani, on the seed surface in spite of their high contamination level.

We also checked seed germination as an indicator of seed quality. The germination of plasma-treated seeds was found to be delayed, though the germination rate reached to 70% at 6 days after treatment (Table 1). Because the treated area of the atmospheric plasma apparatus used in this study is small and the generated plasma was concentrated in a narrow well, the temperature around the seed may have been too high. The microplate well internal temperature was checked with a temperature indicator (Thermolabel Super Mini 3R-70 and 80, Nichiyu Giken Kogyo Co., Ltd., Saitama, Japan) during the plasma treatment. The label indicated that the temperature rose to 75°C after a 2 min treatment, and it reached 80°C within a 5 min treatment. The heat from the plasma discharge might have damaged the seeds in the well.

For the low pressure gas plasma treatment, 15 of the seed samples were set on a mesh sheet in the plasma apparatus (Fig. 1). The plasma was generated by AC high voltage discharge with argon. The voltage and frequency applied to electrodes were 10 kV and 10 kHz, respectively. The argon gas flow rate was 3L/min. The treatment time and the distance from the plasma source to the sample surface were 10 min and 30 mm, respectively, which were found to be the best conditions for sterilizing R. solani culture disks in our previous study (Nishioka et al., 2013). After the plasma treatment, treated seeds were placed on a new water agar plate for detection of R. solani. The new plates were incubated for 6 days at 30°C and the rate of the seeds with surviving R. solani was considered as the fungal survival rate. This treatment was repeated six times.
In our study gas plasma produced and applied under both types of systems, atmospheric pressure or low pressure, could inactivate the mycelia of R. solani, one of the resting structures, on brassicaceous seeds quickly. Vacuum equipment is not needed to generate atmospheric plasma, thereby making the decontamination process practical and inexpensive (Yu et al., 2007; Yun et al., 2010). For application to seed disinfection, improvement in our system or treatment method is required to suppress the rise in temperature.

The low pressure gas plasma system could inactivate the pathogen without damage, and it would be a more time-saving and useful non-chemical method for seed disinfection than hot air treatment. Selcuk et al. (2008) reported that their self-designed low pressure plasma system could significantly reduce surface fungal contamination of Aspergillus spp. and Penicillum spp. on grains and legumes. They achieved a 3-log reduction of the Penicillum spp. on wheat within 15 min. The running pressure of 0.5 torr in their system was much lower than in our system, and they used air gasses or SF6 as the treatment gas. Because the reactive species vary within the plasma depending on the plasma source, process parameters and process gases (Fröhling et al., 2012), it is difficult to compare inactivation mechanisms of the different plasma sources with those of our system. However, the lower pressure and other gas conditions might improve the inactivation effect in our system.

The inactivation effect on R. solani was dependent on the plasma treatment time. R. solani was found on 83% of the seeds before treatment and the fungal survival rate dropped to 1.7% after 10 min. Plasma treatment for 40 min completely inactivated the R. solani from the seeds (Fig. 2). The germination rate after 2 or 6 days incubation (transformed by arcsine) was compared using multiple comparison tests (performed with SPSS 11.0J for Windows, SPSS Japan Inc., Tokyo, Japan) (Table 2). There was no significant difference found between the untreated and each plasma-treated group (P>0.05).

The temperature on the mesh sheet in the plasma apparatus was checked with a temperature indicator (Thermolabel Super Mini 3R-40~80, and Heat-Label MN-P, R, S, Micron Co. Ltd., U.K.) (Table 3). Because the temperature in the low pressure plasma treatment was lower after 40 min than in the atmospheric plasma after 10 min, it was thought that the germination ability of the seeds was maintained. Kawaradani et al. (2009) reported that a hot air treatment at 77°C for 2 days could not completely inactivate Rhizoctonia solani on Japanese hornwort seeds (Cryptotaenia japonica Hassk.). It is assumed that the temperature was not a significant factor in inactivating R. solani in our study, because the temperature in the low pressure plasma was up to 77°C in 40 min.

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<table>
<thead>
<tr>
<th>Treatment time</th>
<th>Post-treatment time</th>
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<tbody>
<tr>
<td></td>
<td>2 days</td>
</tr>
<tr>
<td>untreated</td>
<td>95.6</td>
</tr>
<tr>
<td>2 min</td>
<td>96.7</td>
</tr>
<tr>
<td>5 min</td>
<td>100</td>
</tr>
<tr>
<td>10 min</td>
<td>93.3</td>
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<tr>
<td>20 min</td>
<td>90.0</td>
</tr>
<tr>
<td>40 min</td>
<td>90.0</td>
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</tbody>
</table>

The average of 4-6 experiments is shown. No significant differences in seed germination were found among untreated and plasma-treated groups both after 2 days and 6 days of incubation (P>0.05).

<table>
<thead>
<tr>
<th>Plasma treatment time</th>
<th>2 min</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>(min-max)</td>
<td>45-54</td>
<td>50-60</td>
<td>60-71</td>
<td>70-71</td>
</tr>
</tbody>
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| Three extensions of each temperature indicator were set on 3 different points on the sheet (center, middle, margin).
The inactivation effects are also thought to depend on the species and organs of fungi. The plant pathogenic fungus used in our study, *R. solani*, has not been investigated in other research on plasma inactivation of microorganisms. This report will contribute to the seed-borne disease control of seeds. In our future studies, applying the system to other kinds of seeds with different sizes or shapes will be evaluated.

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**REFERENCES**


