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Transport of reactive species generated by nonthermal plasma through rice seed husk

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Plasma treatment of seeds has garnered attention for its ability to induce beneficial plant responses, such as enhanced germination [1–10]. Plasma-generated reactive species must penetrate multiple barriers—including the seed coat, cell wall, membrane, and cytoplasm—before reaching the nucleus and triggering biochemical changes. These structures serve as both filters regulating reactive species penetration and sites for chemical reactions. Understanding the mechanisms governing plasma irradiation effects requires quantifying introduced reactive species and correlating them with molecular plant responses. Developing a robust methodology for reactive species transport remains an urgent challenge.

Our research group developed a highly sensitive LC-MS/MS-based method to detect nitrate ions (NO_3^-), a plasma-derived species known to promote germination, with high precision in seeds [11]. Lettuce seeds were irradiated using a scalable dielectric barrier discharge (DBD) plasma electrode at 24 ℃ and 55% relative humidity. After irradiation, 20 seeds were analyzed via liquid chromatography-triple quadrupole mass spectrometry (LC-QqQ MS/MS, Agilent 1260 Infinity II). A distinct peak at 62 m/z in the MS spectra of plasma-irradiated samples was identified as NO_3^-. Multiple-reaction monitoring (MRM) analysis confirmed NO_3^- concentration, which increased proportionally with irradiation duration: 0.08, 0.29, 0.73, 1.69, and 2.78 nmol/FWmg at 0, 10, 30, 60, and 90 s, respectively. The MRM transition selected—62 m/z (NO_3^-) to 46 m/z (NO_2^-)—was based on prior studies demonstrating its effectiveness in identifying nitrate and nitrite species [12]. A one-minute plasma treatment resulted in over a 20-fold increase in NO_3^- concentration compared to control samples. These findings highlight plasma irradiation as an effective method for introducing reactive oxygen and nitrogen species (RONS) into dry seeds, enabling correlation studies between introduced reactive species and omics-level molecular responses.

Additionally, we employed the KI-starch assay to evaluate plasma-generated active species penetration through the rice husk [13]. This technique detects reactive oxygen species (ROS) via a colorimetric reaction:

$$H_2O_2 + 2I^- + 2H^+ \rightarrow I_2 + 2H_2O$$
$$I_2 + I^- \rightarrow I_3^-$$
$$I_3^- + \text{starch} \rightarrow I_3^- \cdot \text{starch}$$

The resulting triiodide-starch complex exhibits a blue-purple color, allowing visualization of ROS penetration. KI-starch was placed 3 mm beneath the SDBD electrode, with an acrylic seed coat holder (1 mm diameter) positioned on its surface. Plasma was generated via pulsed voltage (13 kVpp, 9.6 kHz repetition frequency), with irradiation durations of 1, 3, and 5 minutes.

Comparing ROS penetration at 1- and 5-minute exposures showed a substantial increase in concentration with extended irradiation. Under 5-minute plasma treatment, the maximum optical density (OD) increased, indicating greater ROS penetration. Since discharge power remained constant, accumulated dose contributed to enhanced penetration. In uncoated seeds, the 5-minute irradiation curve showed significantly higher peaks than coated samples, demonstrating increased ROS accumulation on the reagent surface. Conversely, the seed coat effectively limited ROS diffusion despite prolonged irradiation. These findings confirm that plasma-generated ROS can penetrate seed structural barriers, providing insights into optimizing plasma-based seed treatment strategies.

These findings experimentally confirm that plasma-generated ROS can penetrate the seed coat. This presentation will explore active species permeation and its implications for optimizing plasma-based seed treatment strategies.

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