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Evaluation of rice seed coat penetration characteristics of plasma-generated long-lived reactive oxygen species using the KI Starch method

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Plasma irradiation of seeds is known to induce plant responses such as germination enhancement [1, 2]. To elucidate the mechanism of this effect, it is important to establish a quantitative evaluation method for the reactive species introduced into seeds by plasma irradiation. In a previous study, we used LC-MS/MS to establish a method to quantitatively measure the amount of nitrate ion (NO^{3-}), which has a germination-inducing effect as a plasma-induced active species, in seeds [3]. We have previously obtained results supporting the transport of chemical species from the outside to the inside of the seed through the seed coat, based on fluctuations in molecular modifications inside and outside the seed caused by dielectric barrier discharge plasma irradiation. Here, to answer the question of whether plasma-generated active species really penetrate the seed coat and pericarp, which are responsible for protecting the seed interior from the external environment, we analyzed the two-dimensional distribution of plasma-induced chemical species through the seed coat and pericarp using KI-starch gels [4-6], which are used to visualize the two-dimensional distribution of chemical species.

In this experiment, a KI-starch gel-like reagent was used to visualize the two-dimensional distribution of chemical species. The gel-like reagent used here is colored by an oxidation reaction to detect reactive oxygen species. The plasma source was an atmospheric pressure dielectric barrier discharge (SDBD) electrode, which has a proven track record in inducing plant response by plasma irradiation [7]. A voltage of 13 kVpp and a frequency of 9.6 kHz was applied to the electrode and plasma was generated for 5 minutes. The images obtained from the coloration on the gel surface were then analyzed in ImageJ to obtain the Optical Density (OD value), which is the relative chemical species concentration. Possible active species include H_2O_2, OH, and O [6].

Figure 1(a) shows the results of the color reaction after plasma irradiation and Fig. 1(b) shows two-dimensional distribution profiles of relative species concentrations obtained by OD analysis for the cases without (w/o seed coat) and with (w/ seed coat) rice husks, respectively. The color reaction results show that the plasma-induced reactive species reach the surface of the gel and exhibit coloration both with and without the rice husk. In addition, to quantitatively evaluate the active species that penetrated the rice husk, OD analysis was performed from point A to point B in Figure 1. From the two-dimensional distribution profile, the number of active species reaching the gel surface in the case with the hulls decreases compared to the case without the hulls, and the transport efficiency of the species is calculated from the integral ratio of OD values. This means that the plasma-induced chemical species are not completely shielded by the rice husk, and transparent through the husk.

References
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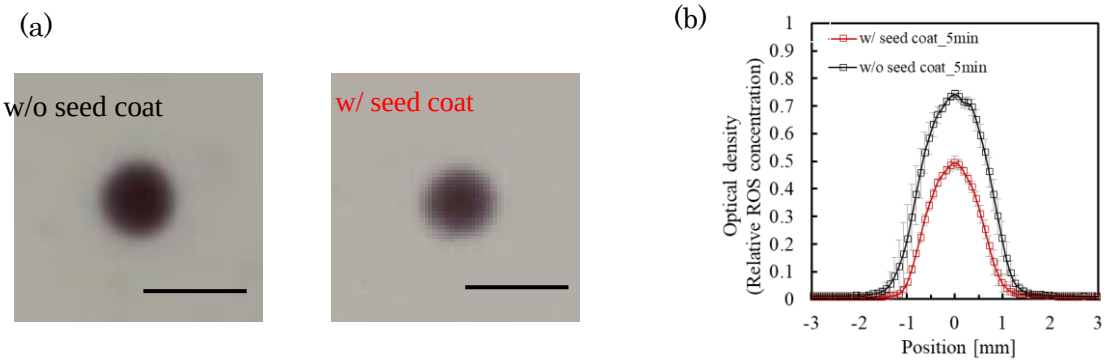

Figure 1. (a)KI-starch gel after plasma irradiation and (b)Relative ROS concentration profiles w/ and w/o seed coat. The scale bar in Fig. 1(a) shows 3mm.