Effect of plasma treatment on growth and harvest of rice seeds

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Abstract: We treated rice seeds with ultrahigh electron-density plasma in order to investigate the optimum condition for growth enhancement. The seeds were getting inactivated for long time more than 10 s. On the other hand, the elongation of coleoptiles was promoted with the treatment for less than 10 s, in a range of small influence on germination. Moreover, we cultivated the seedlings in the growth chamber, those from the plasma-treated seeds headed earlier by 11.7 days at maximum than control ones.

Keywords: Rice seeds, Direct irradiation, Growth promotion

1. Introduction

Recently, various application of non-equilibrium atmospheric-pressure plasma (NEAPP) in the biological fields, such as medicine, agriculture, and fishery, has much attentions. Plant growth consists of multiple steps, such as seeds, seedlings, flowerings, and harvest of fruits. Therefore, for the agricultural applications, various uses can be expected such as growth enhancement, increase of yields, and inactivation of pathogenic microorganisms. The growth enhancement with plasma treatment have been reported for various plant species, such as radish [1-3], *Arabidopsis thaliana* [4], and barley [5].

In our group, we previously developed the ultrahigh electron-density plasma source [6, 7] and reported various biological applications by using the plasma source. *Penicillum digitatum* spores were efficiently inactivated.[8] Besides, for medical application, cancer cells were selectively induced to the apoptotic death with direct treatment.[9] Plasma simultaneously produces various factors such as UV radiation, reactive oxygen species (ROS), electrons and ozone. Those results suggested that ROS particularly affected the spores and cancer cells among the factors. Moreover, we developed the oxygen radical source (Tough Plasma; FUJI Corporation), which can only produce neutral radical species. We can quantitatively estimate the effects of oxygen radicals on the growth of budding yeast cells[10]. According to the increase of oxygen radical doses, the growth was promoted and suppressed, and finally cells were inactivated. Those results suggested that we can regulate both promotion and inhibition of the growth for various species by modulating the intensity of the plasma treatment.

In this study, we irradiated the rice seeds (*Oryza sativa*) with cold plasma during the germination, and we investigated the growth promotion from plasma-treated seeds. Moreover, we prepared for the growth chamber, where various environmental factors for cultivation can be controlled, such as temperature, humidity and day length. We observed the growth of adult plants and investigated the effects on yields.

2. Experimental

Rice seeds were immersed in distilled water at 4°C overnight. Then, two seeds for each treatment were set at 10, 15, or 20 mm below from the nozzle of the plasma source, and treated them for 3, 5, 10, 15, or 20 s. The condition of plasma irradiation was at 2 slm of the flow rate of Ar gas in the air. For second treatment, the plasma irradiations were further performed on the following day. For each condition, more than 36 seeds were prepared at least. The seeds were immersed in distilled water after the plasma treatment and incubated under shade at 30°C for 2 days. And then, we counted both number of germinated and ungerminated seeds to calculate the germination rate. Besides, we measured the length of coleoptile for the growth check after the germination.

Each seedling was sowed on 9 cm-ϕ pot and cultivated in the growth chamber, which is called "Plasma Smart Plant", till harvest. In vegetative-growth period, plants were grown under long-day condition (day length; 14 h). After that, to induce formation of young panicles and heading, we changed the short-day condition (day length; 10 h) in reproductive-growth period.

3. Results and discussion

Figure 1 shows the results of germination rates. After the plasma treatment with rice seeds on various conditions of exposure distances and treatment time at 10, 15, or 20 mm for 3, 5, 10, 15 or 20 s, the seeds were incubated at 30°C for 2 days. Then we measured the germination rates. Almost all of control seeds were germinated. At 10 mm distance, the germination rates gradually decreased till 10 s of treatment time to be ~80%. Next, the rates drastically decreased to be less than 40% with the treatment for more than 15 s. At 15 and 20 mm of exposure distances, the germination rates were higher than those at 10 mm. Nevertheless, the tendencies of the variation were similar with those at 10 mm. The germination rates were ~80% with the treatment for 5 and 10 s, and then drastically decreased for more than 15 s. These results suggested that the embryo cells were inactivated with the plasma

treatment for long time, such as 15 and 20 s. Therefore, we focused on the short-time treatment, such as 5 and 10 s, as conditions without inhibition of germination, in order to investigate the optimal condition for the growth enhancement of rice after germination.

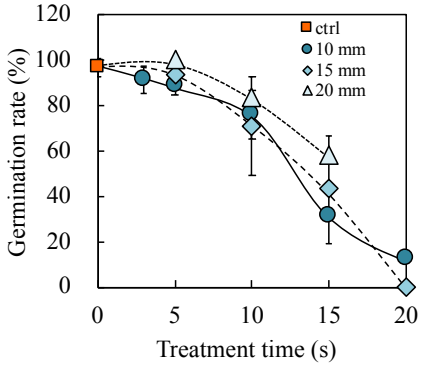

Fig. 1. Variation of germination rate as a function of treatment time at various exposure distances.

Next, we measured the length of coleoptiles in order to estimate the effects on the growth after germination. Figure 2 shows the results of measurement of coleoptiles. The length of coleoptiles from control seeds were ~5 mm after the incubation for 2 days. The coleoptiles derived from the seeds with plasma treatment for 5 and 10 s at 10 mm distance significantly became +80% and +87% longer than those from control seeds, respectively. The results indicated that the plasma treatment with rice seeds promoted the growth after germination. Moreover, in the case of seeds with two treatments for 5 s (10 mm, 5 s ×2), the elongation was further significantly promoted to be +13% compared with one treatment. These results indicated that the degree of the promotion was dependent on the frequency of the treatment. The results suggested that short time treatment, such as 5 and 10 s, act as moderate stimulation and the embryo cells were activated.

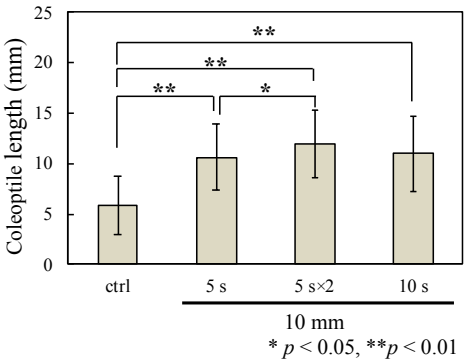

Fig. 2. Length of coleoptiles with plasma treatment under various conditions.

Then, the seedlings from the control seeds and plasma-treated seeds as mentioned above were cultivated in two Plasma Smart Plants till the harvest. In each Plant, we differently changed the day length from long-day condition to short-day condition at 10 and 13 weeks after sowing, which describe early and regular conditions, respectively. ("Regular" condition simulates the whole cultivation period in the field.) Figure 3 shows the number of days from sowing to heading under short and regular conditions. Under early condition, the seedlings of all conditions headed at almost same date. On the other hand, under regular condition, the number of days from plasma-treated seeds were less than that in control ones, which depended on the treatment condition. The plants from the 10 s-treated seeds headed 11.7 days earlier than control. The results suggested that the sensitivity of the plants from plasma-treated seeds to the day length was varied.

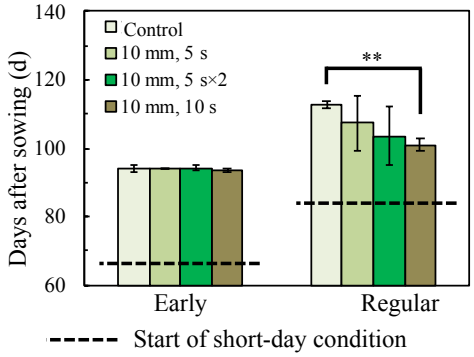

Fig. 3. Number of days from sowing to heading under different day-length conditions.

4. Conclusion

We treated rice seeds with ultrahigh-electron density plasma. The growth from the treated seeds after germination was effectively promoted with short time treatment such as 5 or 10 s. Besides, the promotion degree was dependent on the frequency of the treatment. The results suggest that the short-time treatment acted as moderate stimulation and embryo cells were activated. Then, the seedlings were cultivated in Plasma Smart Plant which can regulate the environmental factors. The plants from plasma-treated seeds headed earlier than control ones. The results suggest that the sensitivity to the day length was varied with plasma irradiation to seeds.

Acknowledgement

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