

Original Article

Seed germination and seedling growth of rice in response to atmospheric air dielectric-barrier discharge plasma

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Abstract

The effect of atmospheric air dielectric barrier discharge plasma on seed germination and early growth has been investigated in rice. Rice seeds were exposed to plasma for 10, 30 and 60 s. It is shown that plasma treatment had positive effects on germination of rice seeds. Seeds exposed to plasma for 60 s had the highest germination rate. Vigor index and germination speed of treated seeds were higher in comparison to untreated seeds. Characteristics of seedling growth, including shoot length, fresh weight and dry weight of shoot and roots were improved by plasma treatment. The contents of photosynthetic pigments tended to increase in the seedlings from treated seeds. In addition, the surface of rice seeds was modified by treating with plasma. These results suggest that the exposure of rice seeds to plasma has the potential to promote seed germination and seedling growth by changing the surfaces of rice seeds.

Keywords: plasma, seed germination, seedling growth, photosynthetic pigment, seed surface

1. Introduction

Rice is one of the most important economic plants in Thailand and is the staple food for more than half of the world's population. The current global increases in temperature are causing declines in seed germination and in growth of various agricultural plants, including rice.

Seed germination is a physiological process starting with water uptake by the dry seed and ending with radicle protrusion, and could determine seedling growth and yield. Temperature affects seed germination through effects on moisture, hormone production and enzyme activities. There are several methods for promoting seed germination, both physical methods (scarification, stratification, magnetic

treatment, sunlight, ultraviolet light and hot water soaking) and chemical methods (chemicals, fungicides and plant growth regulators) (Li *et al.*, 2014). These methods, however, are labor-intensive, time-consuming and leave chemical residues.

Plasma technology is a fast-acting environmentally friendly method and causes little damage to biological materials. It has recently been used in agricultural applications. Previous studies have reported that plasma, an ionized gas composed of neutral molecules, electrons, and positive and negative ions, can improve seed performance, stimulate plant growth, increase crop yield and inactivate microorganisms (Jiang *et al.*, 2014b; Henselova *et al.*, 2012; Sera *et al.*, 2008). The observations of Jiang *et al.* (2014a) demonstrated that seed germination of wheat was improved by treatment with 80 W cold plasma for 15 s. Exposure to air plasma treatment with 5950 V for 10 s could enhance seed germination and seedling emergence of *Andrographispani-*

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culata (Tong *et al.*, 2014). Shoot length, shoot dry weight, root length and root dry weight of soybean seedling were increased by cold plasma treatment (Li *et al.*, 2014). Furthermore, modifications of seed coat structure were observed in the plasma treated seeds. Cold radiofrequency air plasma treatments (10 MHz, 20 W and 6.7×10^{-2} Pa from 15 s to 2 min) changed seed surface properties, including wettability of lentils (*Lens culinaris*), beans (*Phaseolus vulgaris*) and wheat (*Triticum species C9*) (Bormashenko *et al.* 2012). Therefore, in the present study, we determined if plasma treatment promotes the germination of rice seeds. Also the growth response in rice seedlings to treatment of the seeds was investigated.

2. Materials and Methods

2.1 Plasma device

Plasma in these experiments was generated by means of Dielectric-Barrier Discharge (DBD) with air at atmospheric pressure as the medium. The DBD device consists of two parallel planar electrodes made of stainless steels and a High-Voltage (HV) power supply unit, which consists of a frequency generator, a power amplifier, and a car-ignition coil. The HV power supply is operated with a frequency of 5.5 kHz at 18 kV.

2.2 Plant material and plasma treatment

Seeds of rice (*Oryza sativa* L. 'KhaoDawk Mali 105') were obtained from a local farm in Surin, a northeastern province of Thailand. Then, seeds without noticeable defects were selected. The correct term in botany for rice seed is caryopsis, which is the fruit typical of the family Gramineae (rice, corn and wheat). In this study, the term seed is used for convenience.

Rice seeds were spread on the bottom of a glass Petri dish (10 cm in diameter) so that they were not touching each other. The Petri dish with seeds was put on the lower electrode of the device. After that, the seeds were homogeneously treated with plasma discharge generated in the air for 10, 30 or 60 s. The rice seeds without plasma treatment served as the control.

2.3 Seed germination

Seeds with and without plasma treatment were allowed to germinate in laboratory conditions. Three replicates of 100 seeds for each treatment, including the control, were placed on tissue paper in plastic germination boxes and 15 ml of distilled water was added. The germination boxes were incubated at 25 °C for 7 days and the number of germinated seeds was recorded every day. Germination of a seed was called when the radicle had protruded 1 mm or more. The germination percentage was calculated as follows:

$$\text{Germination percentage (\%)} = \left(\frac{\text{number of germinated seeds}}{\text{total number of seeds}} \right) \times 100$$

Vigor index and speed of germination were calculated according to Vashisth and Nagarajan (2010):

$$\text{Vigor index} = \text{germination percentage} \times \text{seedling length (shoot + root)}$$

$$\text{Speed of germination} = \frac{\Sigma(\text{number of seeds newly germinating at time } t / t)}{\text{after sowing}}$$

2.4 Seedling growth

One-week-old seedlings from the alternative treatments were transplanted into pots containing a hydroponic solution (WP) (Vajrabhaya & Vajrabhaya, 1991). The rice seedlings were cultured in the WP solution for 4 weeks, and the growth of seedlings was measured in terms of shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight. WP solution was added to the pot when necessary, to maintain sufficient nutrients for seedling growth.

In addition, photosynthetic pigment contents were measured 4 weeks after transplanting. The contents of photosynthetic pigments were assayed by the spectrophotometric method of Lichtenthaler and Buschmann (2001). Briefly, pigments were extracted from 25 mg of rice leaves in 10 mL of 80% acetone solution. After keeping in the dark for 24 hours, absorbance was measured at the wavelengths 663.2, 646.8 and 470 nm. Then, chlorophyll a, chlorophyll b and carotenoid contents were estimated from the following equations:

$$\text{Chlorophyll a (C}_a\text{)} = (12.25A_{663.2} - 2.79A_{646.8})/\text{FW}$$

$$\text{SChlorophyll b (C}_b\text{)} = (21.5A_{646.8} - 5.10A_{663.2})/\text{FW}$$

$$\text{Carotenoid} = [(1000A_{470} - 1.82C_a - 85.02C_b)/198]/\text{FW}$$

The contents of chlorophyll a, chlorophyll b and carotenoid are expressed in mg/g FW (FW = fresh weight of rice leaves)

2.5 Seed surface structure

After the plasma treatments, three seeds from each treatment, including control, were randomly sampled to examine the surface structure of seeds. They were imaged by scanning electron microscopy (SEM; JSM-5410LV).

2.6 Statistical analysis

The experiment was performed with 3 replicates. The data were subjected to statistical analysis using one-way ANOVA at significance level 0.05, and comparisons between means were conducted by Duncan's Multiple Range Test, using SPSS for Windows.

3. Results

3.1 Effects of plasma treatment on seed germination

Exposure to DBD plasma had positive effects on the germination of rice seeds. Plasma treated seeds showed an increased percentage of germination in comparison to non-

treated seeds. Plasma treatment for 60 s resulted in the maximum percentage of germination (92.67%) that was significantly higher than in the control group (85%) (Figure 1A). However, the plasma treatments for 10 s or 30 s seemed to have similar effects on germination, although without significant differences to the other treatments.

The vigor index and germination speed of rice seeds were enhanced by plasma treatment. The results demonstrate that exposure of seeds to plasma significantly increased the vigor index and the speed of germination, except for the shortest 10 s plasma treatment (Figure 1B and 1C). The increases were consistent in a dose-dependent manner.

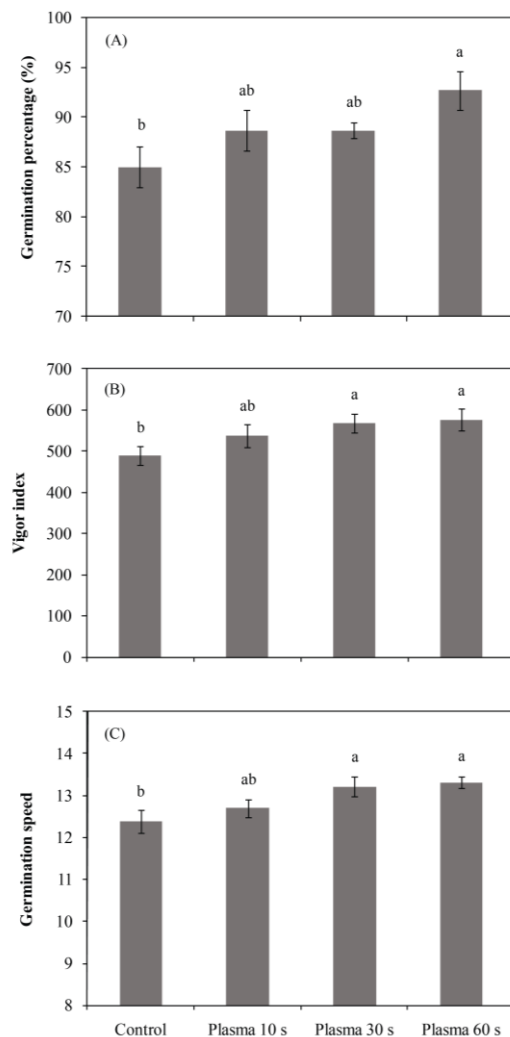


Figure 1. Germination percentage (A), vigor index (B) and germination speed (C) of rice seeds with alternative treatments. Bars with different letters are significantly different ($P < 0.05$).

3.2 Effects of plasma treatment on seedling growth

The characteristics of seedling growth improved as a result of plasma treatment. The 4 weeks old seedlings derived from plasma treated seeds showed longer shoot lengths

than in the control group. Statistically significant increases in shoot length were observed already in response to plasma treatment for 10 s (Table 1). However, the plasma treatments of rice seeds had no effect on the length of roots (Table 1).

Shoot and root fresh weight of treated rice seedlings seemed to be higher than those of control seedlings, but without statistically significant difference (Table 1). In contrast to fresh weight, dry weight of both shoot and roots of plasma treated seedlings were significantly increased when compared to non-treated seedlings (Table 1). Seeds exposed to plasma for 30 s or 60 s produced the most seedling mass.

The contents of photosynthetic pigments, namely chlorophyll a, chlorophyll b and carotenoid, were influenced by plasma treatment. Chlorophyll a and carotenoid content tended to increase in seedlings from treated seeds when compared to those in the control group, but without significant difference (Table 2). Exposure of rice seeds to plasma also increased the content of chlorophyll b in seedlings, with a significant increase observed in response to plasma treatment for 60 s (Table 2).

Table 1. Growth parameters of 4 week old rice seedlings from seeds subjected to various treatments.

Treatment	Length (cm)		Fresh weight (g)		Dry weight (g)	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	48.00±1.27b	8.22±0.48a	2.932±0.062a	1.529±0.043a	0.542±0.013b	0.152±0.004b
Plasma 10s	53.03±1.47a	7.93±0.50a	3.033±0.035a	1.495±0.030a	0.540±0.012b	0.154±0.005b
Plasma 30s	51.87±1.27ab	7.57±0.44a	3.055±0.106a	1.600±0.046a	0.551±0.014ab	0.171±0.003a
Plasma 60s	50.68±2.03ab	8.00±0.60a	3.083±0.082a	1.531±0.074a	0.582±0.008a	0.159±0.013ab

Notes: Data are expressed as mean ±SE. Means with different letters within a column are significantly different ($P < 0.05$).

Table 2. Photosynthetic pigment contents of 4 week old rice seedlings grown from seeds subjected to various treatments.

Treatment	Pigment content (mg/g FW)		
	Chlorophyll a	Chlorophyll b	Carotenoid
Control	0.325±0.016a	0.146±0.005b	0.144±0.004ab
Plasma 10s	0.321±0.035a	0.154±0.016ab	0.145±0.003ab
Plasma 30s	0.315±0.036a	0.145±0.013b	0.139±0.014b
Plasma 60s	0.343±0.019a	0.161±0.010a	0.155±0.004a

Notes: Data are expressed as mean ±SE. Means with different letters within a column are significantly different ($P < 0.05$).

3.3 Effects of plasma treatment on seed surface

The SEM images of rice seeds demonstrate that the surface of rice seeds consisted of pointed conical elements that were well aligned. Small needlelike structures were ran-

domly distributed throughout the seed surface. Exposure of rice seeds to plasma resulted in modifications of surface structure (Figure 2). Plasma treated seeds had smoother surfaces than non-treated seeds. With increasing exposure, there was a progressive decrease in roughness of seed surfaces.

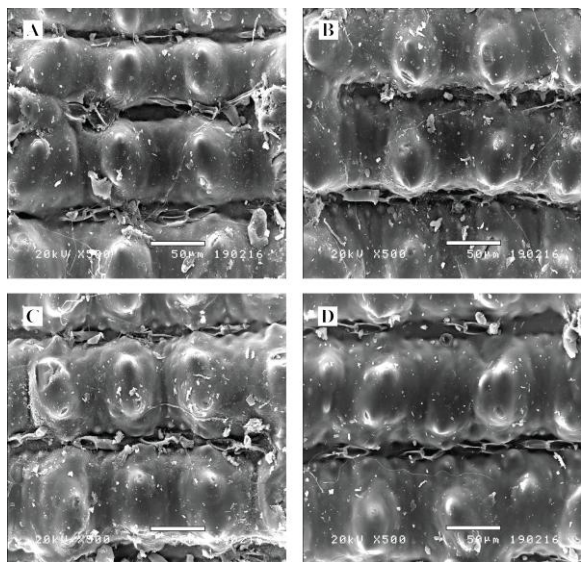


Figure 2. SEM images of rice seed surfaces without (A) and with plasma treatment for various durations (B= 10 s, C= 30 s and D= 60 s). The scale bar represents 50 µm.

4. Discussion and Conclusions

Exposure of rice seeds to various durations of DBD plasma significantly increased germination-related characters, including germination percentage, speed of germination and vigor index, in a dose-dependent manner. These results are consistent with a previous study in soybeans where seed germination and vigor index increased after cold plasma treatment (Li *et al.*, 2014). Bormashenko *et al.* (2012) reported the modification of germination speed in lentils, beans and wheat C9 by cold radiofrequency air plasma treatment. Germination rate of wheat seed was also found to increase in response to plasma treatment (Jiang *et al.*, 2014a).

Numerous studies have reported that plasma treatment promotes seedling growth. In this study, positive effects of plasma treatment on the growth of rice seedlings were observed. This is in agreement with Kitazaki *et al.* (2014) who found that the growth of radish sprouts was enhanced by treating with atmospheric dielectric barrier discharge plasma for 180 s. As a result of cold plasma treatment, shoot length, shoot dry weight, root length and root dry weight of treated soybean seedlings were significantly higher than those of the control seedlings (Li *et al.*, 2014). Moreover, plasma exposure has the potential to improve yield in several plants. For instance, to application of plasma to tomato seeds increased tomato yield and also affected the botanical properties of the plants, including plant height, root length, fruit diameter and fruit weight (Zhou *et al.*, 2011).

SEM images taken from plasma treated and non-treated seeds allowed assessing the seed surface structure.

Clearly the surfaces of rice seeds were modified in response to plasma treatment, in a dose-dependent manner with exposure time. Three possible mechanisms have been proposed to explain the effects of plasma treatment on seed surfaces: etching, surface functionalization and deposition of small bioactive molecules (Zivkovic *et al.*, 2004). During the plasma treatment the seeds may be attacked by oxygen radicals and bombarded by ions, leading to the erosion of seed surfaces. A prior study demonstrated that the surface of cold air plasma treated seeds was enriched with oxygen containing functional groups (Bormashenko *et al.*, 2012). Changes in the surfaces of plasma treated seeds could increase hydrophilic wettability of the seed, leading to accelerated water uptake.

A number of studies have suggested that increased seed germination and seedling growth in response to plasma might be related to water uptake. According to Bormashenko *et al.* (2012), cold plasma treatment modified the wetting properties of the surfaces of lentils, beans and wheat, and eventually increased their germination speed. The positive effects of plasma treatment in stimulating seed germination and seedling growth of soybeans were a consequence of water uptake improvement (Li *et al.*, 2014). It has been suggested that an increase in seed permeability is associated with improved ability to absorb nutrients, which could promote seedling growth.

Furthermore, the activities of seedling germination enzymes might be improved when the cells interact with plasma, leading accelerated nutrient decomposition in the seeds. This might account for increased seed reserve utilization and seedling growth (Dobrynin *et al.*, 2009). Previous studies found that seed reserve utilization of soybeans was significantly increased by cold plasma treatment (Li *et al.*, 2014). The positive impact of plasma on seed performance and plant growth may be at least partially associated with antifungal and antimicrobial activities of the plasma (Kordas *et al.*, 2015; Selcuk *et al.*, 2008). Another possible explanation for the growth stimulation is that the plasma particles (ions, radicals and photons) may stimulate the secretion of hormones; auxin and gibberellins. These hormones then enhance growth of the plants (Achard *et al.*, 2008; Pasternak *et al.*, 2005).

Therefore, it can be concluded that the atmospheric air DBD plasma has potential for improving seed germination and seedling growth. The positive effects of plasma treatment could be associated with the modification of seed surface. In addition, the enhancements of seed germination and seedling growth suggest that plasma treatment may affect rice yields and could be used in future rice production. However, more experiments are necessary to examine the plasma effects on rice yields, and to provide some information on how the plasma treatment promotes rice seed germination and seedling growth.

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