# Effect of Cold Plasma Treatment on Seed Germination and Growth of Wheat*

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**Abstract** This study investigated the effect of cold helium plasma treatment on seed germination, growth and yield of wheat. The effects of different power of cold plasma on the germination of treated wheat seeds were studied. We found that the treatment of 80 W could significantly improve seed germination potential (6.0%) and germination rate (6.7%) compared to the control group. Field experiments were carried out for wheat seeds treated with 80 W cold plasma. Compared with the control, plant height (20.3%), root length (9.0%) and fresh weight (21.8%) were improved significantly at seedling stage. At booting stage, plant height, root length, fresh weight, stem diameter, leaf area and leaf thickness of the treated plant were respectively increased by 21.8%, 11.0%, 7.0%, 9.0%, 13.0% and 25.5%. At the same time, the chlorophyll content (9.8%), nitrogen (10.0%) and moisture content (10.0%) were higher than those of the control, indicating that cold plasma treatment could promote the growth of wheat. The yield of treated wheat was 7.55 $t \cdot ha^{-1}$, 5.89% more than that of the control. Therefore, our results show that cold plasma has important application prospects for increasing wheat yield.

**Keywords:** cold plasma, seed treatment, wheat, seed germination, plant growth, increased yield

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(Some figures may appear in colour only in the online journal)

## 1 Introduction

According to the United Nations Food and Agriculture Organization (FAO), world grain production was about 2.216 billion ton, while the consumption reached 2.254 billion ton in 2010, and there were still 9 million people in hunger. Therefore, food security of the world, especially in China, accounting for 22% of the world population, has become the most severe problem all over the world. In China, the per capita arable land was 40% the level of the world average, and was still decreasing. With the fast development of industrialization and urbanization, requirements for grain yield and grain quality are confronted with new challenges. It is the only way to secure food safety by improving per unit yield of crops in view of the fact that the cultivatable land is hard to increase.

The traditional method to improve crop yield is to improve the fertilization [1] and irrigation [2]. However, these measures are always subject to economic [3] and environmental [4] conditions, and their effects are not sustainable [5]. Significant advances have been made in the area of genomics over the past ten years. Genome sequences are available now for many crop species such as rice. Molecular breeding [6] and genetic engineering [7] are new ways to improve the yield of crops. However, breeding for abiotic stress tolerance is constrained by the complex nature of abiotic stress tolerance, and it is hard to keep its quantification and repeatability. Genetic engineering could bring ecological risk worldwide. As a fast, cheap, green and riskless method, the application of cold plasma technology in agriculture provides a new path for improving grain yield.

Cold plasma seed treatment is a modern eco-agricultural high-tech that could increase crop yields. It is quite different from space breeding or mutation breeding by particle beam. Based on non-ionizing low level radiation, it could activate the vitality of seed without gene mutation, so there is no genetic risk. Zivkovic [8] has demonstrated that cold air plasma pre-

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treatment could significantly improve the germination of *Paulownia tomentosa*. Carvalho [9] showed that thin films obtained by plasma polymerization could protect grains and seeds and enhance seed germination. It was reported that magnetized plasma treatment could increase the germination percentage and peroxidase activities of tomato [10].

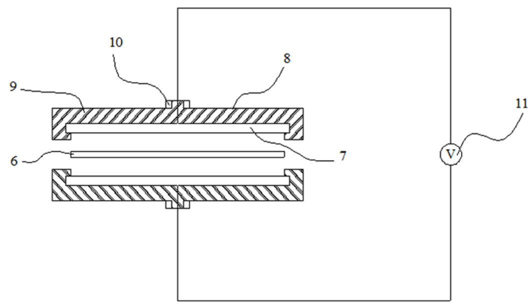
In China, much work has been done on the application of plasma technology in agriculture. Wu [11] has reported the effect of plasma ion implantation on pea seed. It was found that tomato yield was increased after treated with electromagnetic field plasma [12]. Similarly, Yang [13−16] also demonstrated that the growth and yield of lettuce, cucumber, tomato, *Andrographis paniculata* were improved after treated with atmospheric pressure plasma.

Since research on the effect of cold helium plasma on wheat is rare, the objective of the present study is to investigate the effect of cold helium plasma on the growth of wheat, involving: **a.** investigation of the effect of different power of cold helium plasma on wheat seed germination; and **b.** identification of the effect on the growth and yield of wheat influenced by cold helium plasma.

# 2 Materials and methods

## 2.1 Experimental apparatus

The key factor of the cold plasma processing system is cold plasma generator. The device was composed of two parallel plates, which were connected to the radiofrequency power source output. Each plate was provided with a metal suspension shell, and it was filled with insulating materials between the plate and the metal shell. Distance between the two polar plates was 1.5-10 cm. The seed received the non-ionizing radiation treatment when they were conveyed through the cavity between the two polar plates. The configuration of the experimental apparatus is shown in Fig. 1.




6 Conveyer belt, 7 Plate, 8 Metal suspension shell, 9 Insulating materials, 10 Plate joint, 11 Radiofrequency source

**Fig.1** A general view of the experimental apparatus

Process of seed treatment by the cold plasma can be described as:

input → pretreatment → vacuum pumping → cold plasma treatment → output

## 2.2 Seed material

Seeds of wheat (*Triticum* spp.) were collected by Jiangsu provincial agricultural reclamation and development corporation, and only ripe intact seeds without visible defects were selected.

## 2.3 Plasma treatment

Wheat seeds were exposed to plasma discharge under the following parameters: the plasma frequency was $3\times10^9$ MHz, the photon energy was approximately 13 eV, the power was 60 W, 80 W and 100 W, the pressure was adjusted by helium to $P = 150$ Pa after vacuumed, the distance of two plate was 2 cm, the processing time was 15 s. Meanwhile, the control samples were left untreated.

## 2.4 Seed germination test

Seed germination test was based on the rules for agricultural seed testing—Germination test (GB/T 3543.4-1995) of national standard of the People's Republic of China. Each culture dish was covered with two layers of filter paper, and 10 mL of distilled water was added. 50 seeds were placed in each culture dish evenly. The dish was cultured in 25 °C light incubator for 7 days, and the number of seed germination was observed and recorded every day. The standard to determine seed germination is checked when the germ length reaches half of the seed length. The experiment repeats 4 times.

Germination potential (%) = 3d number of germinated seeds / total seed number for testing ×100%

Germination rate (%) = 7d number of germinated seeds / total seed number for testing ×100%

Germination index $G_i = \Sigma(G_t/D_t)$, $G_t$ is the number of germinated seeds at $t$ day, $D_t$ is germination days.

## 2.5 Field experiment

As cold plasma treatment with 80 W could improve the germination potential and germination rate of wheat, we carried out field experiments to study the effect of cold plasma treatment on wheat growth. A field of approximately 12 ha was selected for our experiment at Jiangsu Xinyang farm. The field was flat, middle level fertilized and with homogeneous land fertility. The field was divided into two parts. One part was planted with treated seeds, while the other part was planted with untreated seed. The two parts of the field had the same fertilization and field management. Seeds were sown at October 28, 2012.

20 plants were selected randomly and were analyzed for plant height, root length and fresh weight at January 5, 2013 when the plant was at seedlings stage. Similarly, we analyzed 20 plants randomly for plant height, root length, fresh weight, stem diameter, leaf area and leaf thickness at April 7, 2013 when the plant was at booting stage, at the same time chlorophyll, nitrogen and moisture content were also measured. Approximately 1500 m$^2$ field was randomly se-

lected for yield determination at June 15, 2013, and there were three repeats for both the treated and the control groups, respectively.

## 2.6 Statistical analysis

Data were subjected to analysis of variance (ANOVA), and the least significant differences at $P<0.05$ (Fisher LSD) was determined. Analyses were performed using the Statistics software package (SPSS 18.0).

# 3 Results

## 3.1 Seed germination

The effect of the cold plasma on seed germination varied with the power of treatment (Table 1). Germination potential and germination rate of control were 84.0% and 83.3%, respectively, and there were no significant differences between treatment with 60 W, 100 W and control in germination potential and germination rate. Treatment of 80 W could significantly increase germination potential (6.0%) and germination rate (6.7%) of wheat seeds. However, germination index of all treatments had no significant differences compared to the control.

**Table 1.** Effect of treatment with different power on seed germination

| Treatment | Germination rate(%) | Germination potential(%) | Germination index |
|---|---|---|---|
| CK | 84.0±2.0a | 83.3±1.2a | 20.9±1.7a |
| 60 W | 84.4±2.1a | 82.7±2.3a | 20.3±0.2a |
| 80 W | 90.7±3.0b | 88.3±2.9b | 21.6±1.2a |
| 100 W | 84.0±3.4a | 84.3±0.6a | 20.6±0.2a |

The data were expressed as the mean ± standard deviation (SD). Different letters within a column indicate significant differences as determined by the LSD test ($P$=0.05)

## 3.2 Plant growth

Plant height of treated wheat seedling was 14.8 cm, significantly higher than that of the control, which was 12.3 cm. Treated plant also had longer root length (8.5 cm) compared with the control (7.8 cm). In addition, fresh weight of treated plant was significantly higher than that of the control by 21.8% (Table 2).

At booting stage, plant height, root length, fresh weight, stem diameter, leaf area and leaf thickness of the treated plant were 60.2 cm, 15.1 cm, 26.90 g, 4.61 mm, 86.9 $cm^2$ and 0.359 mm, significantly increased by 21.8%, 11.0%, 7.0%, 9.0%, 13.0% and 25.5%, respectively, as compared with the control (Table 3).

**Table 2.** Effect of cold plasma treatment on the growth of wheat seedling

| | Plant height (cm) | Root length (cm) | Fresh weight (g) |
|---|---|---|---|
| CK | 12.3±1.7a | 7.8±0.9a | 1.10±1.87a |
| Treated | 14.8±1.8b | 8.5±0.9b | 1.34±0.24b |

The data were expressed as the mean ± standard deviation (SD). Different letters within a column indicate significant differences as determined by the LSD test ($P$=0.05)

Table 4 showed that the cold plasma treatment could also influence the physiological activities of wheat. Chlorophyll content of the treated plant was 15.7%, 9.8% higher than that of the control. Meanwhile, there were no significant differences between the treated and the control in nitrogen and moisture contents.

**Table 4.** Effect of cold plasma treatment on physiological metabolism of wheat at booting stage

| | Chlorophyll content (%) | Nitrogen (mg/g) | Moisture content (%) |
|---|---|---|---|
| CK | 14.3±1.3a | 1.0±0.1a | 20.9±2.1a |
| Treated | 15.7±1.4b | 1.1±0.1a | 23.0±1.8a |

The data were expressed as the mean ± standard deviation (SD). Different letters within a column indicate significant differences as determined by the LSD test ($P$=0.05)

## 3.3 Yield of wheat

The yield of the treated wheat was 7.55 $t \cdot ha^{-1}$, 5.89% more than that of the control (Fig. 2). The results showed that cold plasma seed treatment could improve the yield of wheat.

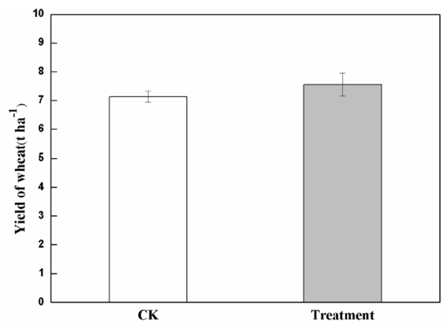



**Fig.2** Effect of the cold plasma treatment on the yield of wheat

**Table 3.** Effect of cold plasma treatment on the growth of wheat at booting stage

| | Plant height (cm) | Root length (cm) | Fresh weight (g) | Stem diameter (mm) | Leaf area ($cm^2$) | Leaf thickness (0.01 mm) |
|---|---|---|---|---|---|---|
| CK | 49.4±3.0a | 13.6±1.0a | 25.13±2.44a | 4.23±0.29a | 76.9±4.9a | 28.6±1.0a |
| Treated | 60.2±3.9b | 15.1±1.7b | 26.90±2.84b | 4.61±0.33b | 86.9±5.2b | 35.9±1.4b |

The data were expressed as the mean ± standard deviation (SD). Different letters within a column indicate significant differences as determined by the LSD test ($P$=0.05)

# 4 Discussion

Seed dormancy is an innate seed property that enables the species to reproduce generatively to survive [17]. Cold plasma is known as a recent stimulation treatment to break down dormancy [18]. Plasma treatment could generate UV radiation, radicals and chemical reactions, which played a great role in dormancy breaking. Cold helium plasma might function in dormancy breaking, and it was in accordance with Sera's [19].

Volin [20] reported a significant delay in the germination speed of seeds treated by Fluorocarbon plasmas. Their findings are just the opposite to our results. When they exploited fluorocarbon plasmas, the seed coat characteristics were modified via plasma-deposition of hydrophobic materials, which would decrease water absorption and thus result in delayed germination. In our experiments, the helium plasma was exploited, which might make improvement in the wettability of seeds and eventually influence their germination speed. Sera [21] and Bormashenko [22] also had similar reports.

The effect of plasma treatment with different intensities (output power) was different [23]. Plasma treatment with low energy might be insufficient to bring about obvious effects on seeds. Meanwhile, the effect of plasma treatment with high energy might be too strong so that it wouldn't cause appropriate effects on seeds. Thus, each kind of seeds had its own most suitable processing power, accordingly. On this basis, we carried out seed germination test for wheat treated with different power (60 W, 80 W, 100 W), and it was found that the most appropriate power of treatment was 80 W for wheat seeds.

Previous studies have reported that cold plasma treatment could improve the growth of wheat and oat, with the weight of shoots significantly increased [19]. In the present experiment, we observed that the plant height, root length and fresh weight of wheat seedlings treated by cold helium plasma were significantly improved. Since the hydrophilic of seeds was improved by cold plasma treatment, and the ability of plants to absorb water and nutrition was relatively enhanced, leading to a better growth. Satoshi [24] also reported that cold plasma treatment could improve the growth of radish sprouts.

Our data showed that the treated plant had a better growth than the control at booting stage. Compared to the control, the treated wheat seedlings had longer root, higher height and heavier weight, therefore they were better at absorbing water and nutrition, and could get more light for photosynthesizing. The effect of cold plasma treatment could improve the growth of wheat not only at seedling stage but also at booting stage. At the same time, we found that chlorophyll content of the treated wheat was higher than that of the control, indicating that cold plasma treatment could increase the physiological activities of wheat. This is in agreement with Sera [19] and Henselova [25].

The most concerned is still the yield. Our results showed that the yield of treated wheat was 7.55 $t \cdot ha^{-1}$, remarkably 5.89% more than that of the control. At the booting stage, the treated wheat had longer root, making it more convenient to absorb water and nutrition. And the plant height was higher, so it could get more sunshine. What's more, leaf area, leaf thickness and chlorophyll content of the treated wheat are higher, causing a higher level of photosynthesis. All these good characteristics of treated wheat indicated an increased yield.

# 5 Conclusion

In this study, we firstly reported that cold helium plasma seed treatment could increase the yield of wheat. The cold plasma treatment could improve the germination, promote the growth and increase the physiological level of wheat, leading to increased yield. Our research shows the feasibility and advantages of cold plasma application to seed treatment, and also provides theoretical basis for the utilization and popularization of this technique. Further research is needed to fully understand the specific role of cold plasma treatment in promoting the growth of wheat.

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