Improving Seed Germination and Peanut Yields by Cold Plasma Treatment

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Abstract This study explored the effects of cold plasma treatment on seed germination, plant growth, and peanut yield. Cold plasma treatment improved germination and seedling growth, and the 120 W treatment produced the best effect. Germination potential and germination rate were markedly raised by 150% and 21%, respectively. Germination was accelerated and the uniformity of emergence improved. The apparent contact angle was decreased by 53%. Seedling shoot and root dry weights increased by 11% and 9%. Leaf area, leaf thickness, leaf nitrogen concentration, chlorophyll contents, and dry weight at the fruiting stage, together with plant height, stem diameter, and root dry weight at the mature stage were all markedly raised by the cold plasma treatment. The cold plasma treatment enhanced yield components, such as branch numbers per plant, pod numbers per plant, and 100 pod weights by 8%, 13%, and 9%, respectively, compared to the control. Furthermore, the yield improved by 10%. These results suggested that cold plasma treatment improved germination, plant growth, and yield, which might be due to the cold plasma increasing the leaf area, nitrogen concentrations, and chlorophyll contents.

Keywords: cold plasma, peanut, germination, dry weight, yields

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

1 Introduction

The world population is increasing rapidly, and food and oilseed oil requirements are rising [1]. Peanut (Arachis hypogaea L.) is an important oilseed crop and is now a large feed meal and vegetable oil crop, providing about 30% of the total vegetable oil supplies in China. The planting area and total production of peanut have reached 5 million ha and 15 million metric tons, and account for about 20% and 42% of the world’s production area and yield, respectively [2]. Although China is a world leader in peanut production, the average yield and oil production in China has remained low.

Germination and the early seedling growth stages play very important roles in plant life [3]. The traditional method to improve seed germination is to use magnetic field, ultraviolet, and other physical pathways to irradiate seeds, or to use chemicals, fungicides and hormones to soak the seeds before sowing [4–9]. However, these methods each have their own limitations, such as being time consuming, labor-intensive and environmentally unhealthy [10]. Therefore, there is a need to investigate a new method to improve seed germination and plant production.

Plasma has been widely used in surface modification and medicine [11]. Cold plasma is known as an eco-agricultural technology that plays important parts in plant growth, such as reducing the numbers of Aspergillus, Penicillium, E. coli and B. subtilis that are attached to the surface of seeds [12–14]; it changes the wetting properties and water imbibitions of seeds, and stimulates seed germination, plant growth, and plant yield [15–17]. Cold plasma also changes the physiological metabolism in plants. For example, it improves the activities of α-amylase, lipase,
and protease in seeds [18], accelerates seed reserve utilization in early seedling growth [19], increases chlorophyll contents, and improves photosynthetic capacity and the activities of superoxidase, peroxidase and polyphenoloxidase [20–24]. However, few studies have focused on the influence of cold plasma on seed germination, plant growth and peanut yield.

The objectives of this work were to (1) investigate the effect of different cold plasma treatments on germination, plant growth, and peanut yield; and (2) to understand the mechanisms underlying cold plasma and their effects on plant growth and yield.

2 Materials and methods

2.1 Experimental apparatus and treatment conditions

The experimental apparatus for seed cold plasma treatment was described in our previous study [19]. The experiment was carried out using HD-2N plasma units. Peanut (Arachis hypogaea L. cv. Eyou 7) seed was treated with an inductive helium plasma discharge. The experimental conditions for the cold plasma treatment were as follows: the generator frequency was 13.56 MHz, the pressure was 150 Pa, treatment time was 15 s, and temperature was about 25°. The treatment power was 60 W (T1), 80 W (T2), 100 W (T3), 120 W (T4), or 140 W (T5), and the volume of the discharge chamber was 1200 mm x 180 mm x 20 mm. The non-treated seeds were considered to be a control (CK). A scheme for the experimental apparatus is shown in Fig. 1. The cold plasma treatment process is:

Input → Pretreatment → Vacuum pumping → Cold plasma treatment → Output

Fig.1 Cold plasma apparatus used to treat the peanut seeds. 1 radio frequency generator, 2 inlet hopper, 3 seeds, 4 cold trap, 5 vacuum pump, 6 plates, 7 metal suspension shell, 8 conveyor belt, and 9 outlet hopper

2.2 Germination assay

The test was based on the method according to Li et al [19]. Seed germination was determined by the petri dish method. The seeds were incubated in a constant temperature incubator for 7 days and then seed germination was calculated. Three replicates of 50 seeds were used for each treatment. The seeds were incubated in a 110 mm petri dish on two layers of filter paper in the dark at 25°C in an incubator. Germination potential and germination rate were determined according to Li et al [19]. Seedlings dry weights were measured on the 7th day.

The Richards function and population parameters viability (Vt), median germination time (Mt), dispersion (Qt), and skewness (St) were calculated according to Richards [20] and Bormashenko et al [21]. The Richards function Yt was calculated according to the following equation:

\[
Y_t = \frac{a}{1 + b \times d \times \exp(-c \times t)^\frac{1}{d}},
\]

where a, b, c and d are the fitting parameters, and t is the time.

2.3 Apparent contact angle of peanut seed

The apparent contact angle was determined using a Kino goniometer (model SL200B).

2.4 Yield experiment

A field experiment was conducted from April, 2013 to October, 2014 and was located at the Yingtan Red Soil Research Station, Institute of Soil Science, Chinese Academy of Sciences, Yingtan, China (117°02’E, 28°23’N). The soil (red soil) has a pH of 4.9, and contained 600.1 mg kg⁻¹ dissolved organic carbon, 0.43 g kg⁻¹ total nitrogen, 4.2 mg kg⁻¹ available phosphorus and 137 mg kg⁻¹ available potassium. The experiment contained two treatments: non-treated (CK) and 120 W treatment, and the treatments were arranged in a randomized block design. All seeds were sown on 10 April, 2013. The experiments were replicated three times with a plot size of 56 m², and seeds were sown in rows separated by about 20 cm. Each plot was fertilized with urea (262.5 kg ha⁻¹), calcium magnesium phosphate (750 kg ha⁻¹), and potassium chloride (262.5 kg ha⁻¹) at the beginning of the experiment.

2.5 Plant growth at the fruiting stage

Three plants were chosen at random from each plot to determine leaf area, leaf thickness, soil and plant analyzer development (SPAD) value, leaf water content, and leaf nitrogen (N) concentration. The whole plant was oven dried at 80°C for 48 h to a constant weight to measure the dry sample weight at the fruiting period. The SPAD value, leaf water content and leaf N concentration were measured by a plant nutrient measuring instrument (TYS-3).

2.6 Agronomic traits, yield and yield components

Three plants were selected randomly from each plot to determine the plant height, stem diameter, shoot dry
weight, root dry weight, branch number per plant, pod number per plant, and 100 pod weights. The samples were oven-dried at 80°C for 48 h to a constant weight. At final harvest, a total area of 56 m² was harvested from each plot. The pods were removed from the plants and weighed.

2.7 Statistical analysis

Data were reported as means with standard error. Significant results were analyzed by Duncan’s multiple range tests (Duncan’s test) using SPSS (16.0) via one-way analysis of variance (ANOVA).

3 Results

3.1 Seed germination

The results showed that the influences of different levels of cold plasma treatment on germination were not the same. The germination potential of CK was 13%, whereas the T2, T3 and T4 treatments significantly increased the germination potentials by 128%, 128% and 150%, respectively, compared to CK (Fig. 2). However, the T1 and T5 treatments had no positive effect on the germination potential. T4 treatment significantly increased the germination rate by 21%, compared to CK. T1, T2, T3 and T5 treatment had no obvious influence on the germination rate. The maximum germination potential and germination rate were obtained from the T4 treatment.

The germination data fitted the Richards function well (Fig. 3). The final germination rate (Vi) and skewness (Sk) were almost the same for all cold plasma treated and untreated seeds (Table 1). The values for Me, in the T2, T3, and T4 treatments were lower than for the untreated seeds, which indicated that germination had accelerated. The values for Qu in the T2, T3, and T4 treatments were higher than for the untreated seeds, which indicated that the uniformity of emergence had improved.

3.2 Apparent contact angle

The apparent contact angle of the control was 99.97° (Fig. 4). The T2, T3, T4 and T5 treatments dramatically decreased the apparent contact angle by 28%, 45%, 53% and 36%, respectively, compared to the control. No significant differences in apparent contact angle were found between T1 and the control. The lowest apparent contact angle occurred using the T4 treatment.

![Fig.2](image)

**Fig.2** Influence of cold plasma treatment on peanut germination potential (left panel) and germination rate (right panel). Different letters indicate significant differences at P < 0.05 level among all treatments as determined by Duncan’s test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vi (%)</th>
<th>Me (d)</th>
<th>Qu (d)</th>
<th>Sk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>100.05±0.03</td>
<td>3.74±0.01</td>
<td>0.39±0.01</td>
<td>−0.58±0.03</td>
</tr>
<tr>
<td>T1</td>
<td>100.05±0.03</td>
<td>3.71±0.01</td>
<td>0.38±0.01</td>
<td>−0.57±0.02</td>
</tr>
<tr>
<td>T2</td>
<td>100.65±0.39</td>
<td>3.52±0.01</td>
<td>0.62±0.03</td>
<td>−0.55±0.02</td>
</tr>
<tr>
<td>T3</td>
<td>100.26±0.08</td>
<td>3.50±0.01</td>
<td>0.61±0.02</td>
<td>−0.58±0.01</td>
</tr>
<tr>
<td>T4</td>
<td>100.03±0.01</td>
<td>3.40±0.02</td>
<td>0.55±0.02</td>
<td>−0.60±0.02</td>
</tr>
<tr>
<td>T5</td>
<td>100.03±0.01</td>
<td>3.68±0.01</td>
<td>0.42±0.01</td>
<td>−0.60±0.02</td>
</tr>
</tbody>
</table>

Data are the mean ± standard error (SE) of three replications.
3.3 Seedling growth

Cold plasma treatment improved seedling growth (Fig. 5). The shoot dry weight for the T4 treatment was 46.62 mg, which was 11% higher than CK, but the T1, T2, T3, and T5 treatments had no significant influence on shoot dry weight. The T2, T3 and T4 treatments increased the root dry weight by 4%, 5% and 9%, respectively, compared to CK. The maximum shoot weight and root dry weight were obtained from the T4 treatment.

3.4 Plant growth at the fruiting stage

Cold plasma treatment has a positive influence on plant growth at the fruiting stage (Table 2). The leaf area and leaf thickness of CK was 44.04 cm² and 0.9 mm, which were improved by 15% and 7% by cold plasma treatment, respectively. Leaf N concentration
and SPAD value after cold plasma treatment were 1.40% and 19.62, which were 3% and 9% higher than CK, respectively. The positive effect of cold plasma treatment on dry weight was similar to its effect on the SPAD value. The dry weight increased by 12% compared to CK. However, cold plasma treatment had no significant effect on leaf water content.

### 3.6 Peanut yield

Cold plasma treatment improved peanut yields (Table 4). Yield components such as branch numbers, pod number and 100 pod weights, after cold plasma treatment increased by 8%, 13% and 9%, respectively, compared to CK. The yield of cold plasma treated plants averaged 6577.78 kg·ha⁻¹, which represented a 10% improvement compared to CK.

**Table 2.** Influence of cold plasma treatment on peanut plant growth at the fruiting stage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf area (cm²)</th>
<th>Leaf thickness (mm)</th>
<th>Water content (%)</th>
<th>N concentration (%)</th>
<th>SPAD value</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>44.04±0.26 b</td>
<td>0.90±0.01 b</td>
<td>28.71±1.04 a</td>
<td>1.40±0.01 b</td>
<td>19.62±0.25 b</td>
<td>10.34±0.32 b</td>
</tr>
<tr>
<td>120 W</td>
<td>50.62±1.96 a</td>
<td>0.96±0.01 a</td>
<td>31.22±1.17 a</td>
<td>1.44±0.01 a</td>
<td>21.32±0.52 a</td>
<td>11.60±0.26 a</td>
</tr>
</tbody>
</table>

Data are the mean ± standard error (SE) of three replications. Different letters in the same column indicate significant differences at $P < 0.05$ level as determined by Duncan’s test.

**Table 3.** Influence of cold plasma treatment on peanut agronomic traits at the mature period

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Stem diameter (mm)</th>
<th>Shoot dry weight (g)</th>
<th>Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>44.73±0.64 b</td>
<td>6.92±0.15 b</td>
<td>29.57±0.78 a</td>
<td>1.25±0.01 b</td>
</tr>
<tr>
<td>120 W</td>
<td>47.25±0.58 a</td>
<td>7.64±0.28 a</td>
<td>32.69±1.73 a</td>
<td>1.37±0.04 a</td>
</tr>
</tbody>
</table>

Data are the mean ± standard error (SE) of three replications. Different letters in the same column indicate significant differences at $P < 0.05$ level as determined by Duncan’s test.

**Table 4.** Influence of cold plasma treatment on yield of peanut

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Branch number per plant</th>
<th>Pod number per plant</th>
<th>100 pod weights (g)</th>
<th>Yield (kg·ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td>8.27±0.14 b</td>
<td>22.00±0.67 b</td>
<td>193.98±4.45 b</td>
<td>5970.37±139.71 b</td>
</tr>
<tr>
<td>120 W</td>
<td>8.93±0.23 a</td>
<td>24.92±0.50 a</td>
<td>211.17±6.33 a</td>
<td>6577.78±153.96 a</td>
</tr>
</tbody>
</table>

Data are the mean ± standard error (SE) of three replications. Different letters in the same column indicate significant differences at $P < 0.05$ level as determined by Duncan’s test.
4 Discussion

The cold plasma treatment power is a key factor, since too low a power may not affect seed germination and plant growth, while too strong a power may have detrimental effects on seeds. In this study, cold plasma had a significant effect on seed germination and seedling growth, and the 120 W treatment had the best stimulatory effect (Figs. 2 and 5). This result was consistent with the results of some previous studies about the influence of plasma treatment on germination and seedling growth in several species [11,18,25]. Stolárik et al. [26] revealed that the germination rate of pea seed increased after plasma treatment. A positive effect of plasma on germination was also reported by Dhayal et al. [10] who indicated that lower power plasma treatment markedly stimulated seed germination and seedling growth of safflower. The germination data followed the Richards’ function well (Fig. 3), the speed of germination accelerated, and the uniformity of emergence improved after cold plasma treatment (Table 1). These results were consistent with Bormashenko et al. [21] who reported that germination was accelerated by cold radiofrequency plasma treatment. The mechanisms responsible for cold plasma treatment effects on seed germination are not yet unclear, but some hypotheses have been developed about how cold plasma treatment beneficially affects seeds performance. One of these hypotheses suggests that cold plasma can induce structural changes in the seed coat, leading to an increase in seed wettability, α-amylase and protease activities, and seed reserve utilization by seeds, thus stimulating seed germination and seedling growth [15,27,28].

Many studies have reported that cold plasma treatment influenced the wettability of seed [29–31]. In the present study, cold plasma treatment significantly decreased the apparent contact angle of peanut (Fig. 4), which was in agreement with Bormashenko et al. [17,21] who found that cold radiofrequency plasma decreased the apparent contact angle of lentil, wheat and soybean seeds. Changes in the wettability of seeds are mainly ascribed to the collisions of ions and active oxygen produced by cold plasma [30].

Plasma has been shown to improve plant growth in several plant species [19,31]. Dubinov et al. [32] stated that the dry weights of barley and oats seedlings were increased by glow discharge plasma treatment. The accumulation of biomass of wheat also rose after cold plasma treatment [24]. In accordance with these previously reported results, this study also revealed that the 120 W cold plasma treatment significantly improved leaf area, leaf thickness, leaf water content and dry weight at the fruiting stage and plant height, stem diameter and root dry weight at the mature stage (Tables 2 and 3). The increase in leaf area and leaf thickness after cold plasma treatment may result in a greater interception of light and an increased photosynthetic rate [33]. The increase in root growth after cold plasma treatment may lead to an increase in water and nutrition uptake from the soil.

Chlorophyll is the core component of the plant photosynthesis system. Changes in chlorophyll contents can directly lead to changes in photosynthesis and the accumulation of photosynthetic products [34]. The SPAD value markedly increased after cold plasma treatment (Table 2). This finding suggested that cold plasma treatment stimulated plant growth and yield by increasing the chlorophyll contents.

We found that the total N concentration increased after cold plasma treatment (Table 2). This indicated that the N absorption capacity and utilization efficiency of peanut were increased by cold plasma treatment, which might also explain how cold plasma treatment increased plant growth and yield.

It is known that the branch number per plant, pod number, and 100 pod weights are decisive factors affecting peanut yield [35]. In this study, peanut yield was improved by cold plasma treatment (Table 4). The branch number, pod number per plant, and 100 pod weights increased, which were responsible for the influence of cold plasma treatment on the yield. Similar effects have been reported for wheat [24], grain, and legume yields [14].

5 Conclusions

This study has demonstrated the ability of cold plasma treatment to improve seed germination, plant growth, and yield in peanut. Germination potential, germination rate, and dry weights of seedlings were markedly increased after cold plasma treatment. Under field conditions, leaf area, leaf thickness, and dry weight at the fruiting stage; plant height, stem diameter and root dry weight at the mature stage; and yield were improved by cold plasma treatment. The promotion of plant growth and yield may be associated with the enhancement in chlorophyll content, leaf N concentrations, and the interactions between them. Further studies will investigate the mechanism underlying how cold plasma treatment affects seed germination and yield in peanut.

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