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Influence of low-vacuum helium cold plasma pre-treatment on the rooting and root growth of zoysiagrass (*Zoysia* Willd.) stolon cuttings

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Abstract

The influence of low-vacuum helium cold plasma treatment on the rooting percentage, root growth and physiochemical properties of zoysiagrass stolon cuttings was studied. Zoysiagrass stolon cuttings were pre-treated with 0, 100, 200, 300 and 400 W of cold plasma for 15 s. The cold plasma positively stimulated rooting and improved the root growth of the zoysiagrass stolon cuttings, and the 300 W treatment produced the best effect. The rooting percentage and root growth parameters, including the root length, total root surface area, total root volume, average root diameter, and root dry weight, significantly improved in response to the cold plasma treatment. In addition, the water uptake and relative conductivity of the stolon cuttings increased significantly in response to the cold plasma treatment. The results revealed that cold plasma-stimulated rooting and root growth appear to be a consequence of the improvement in permeability and water absorbing capacity of zoysiagrass stolon cuttings. The results of the present study will provide inspiration and support for the application of cold plasma in the vegetative propagation of plants.

Keywords: cold plasma, zoysiagrass, stolon cutting, rooting, root growth

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

1. Introduction

Plasma is known as the fourth state of matter, comprising excited atoms, molecular, reactive species (reactive oxygen species and reactive nitrogen species, among others), charged particles and electromagnetic radiation [1, 2]. In recent years, cold plasma has been extensively used in polymer engineering, microelectronics, medicine engineering, food processing, waste disposal, and material modification [2]. Cold plasma technology is potentially an efficient, economic and environmentally friendly technology and has recently been widely used in agriculture. Cold plasma treatment is an effective pre-treatment method for seeds, as it stimulates germination and early seedling growth by reducing the amount of microbes [3, 4], promoting both the permeability and water absorbing capacity of seeds [5–8], and stimulating seed reserve utilization [9]. Cold plasma treatment also effectively improves plant growth and crop yields, such as those of wheat [10], peanut [11], and tomato [12, 13].

Turfgrass is an essential component of ecosystems; it increases the aesthetics of the environment, purifies air and water, conserves soil and water, repairs soil, and aids in recreational sports [14]. Zoysiagrass (*Zoysia* Willd.) is an important warm-season turfgrass species that is widely used for home lawns, sports fields, and golf courses in both tropical and subtropical zones, as it has good wear, shade, salt, traffic and drought tolerance and requires little maintenance [15]. Although a few commercial cultivars of zoysiagrass are propagated by seed, most zoysiagrass is propagated vegetatively via stolons,

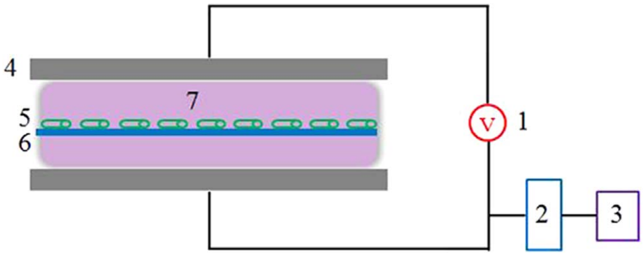

Figure 1. Schematic diagram of the cold plasma device. 1 radio frequency generator, 2 cold trap, 3 vacuum pump, 4 electrode plate, 5 stolon cuttings, 6 conveyer belt, 7 plasma discharge region.

shoots or rhizomes [16]. The vegetative propagation ability depends greatly on a high rooting capacity and rapid rooting. However, the rooting capacity of zoysiagrass stolons is weak, and their rooting and root growth is slow, which significantly slows the speed of turfgrass establishment and increases its cost of establishment [17]. In recent years, the area of zoysiagrass has increased rapidly, which underlies the need to develop economic and effective technologies that can accelerate both the rooting and root growth of this turfgrass species.

The formation of adventitious roots at the nodes of stolons, shoots or rhizomes is an essential step for successful vegetative propagation, which is regulated by various endogenous and exogenous factors [18–20]. As a carrier of physiological and biochemical processes during rooting, water is a key factor involved in adventitious root formation [21, 22]. The water absorbing capacity of vegetative parts is low prior to rooting, and water is continuously lost after vegetative leaves are removed from the mother plant [21]. Therefore, it is essential to increase the water absorbing capacity and water content in vegetative tissue during the rooting process.

The effects of cold plasma pre-treatment on vegetative propagation, including rooting, remain unclear, greatly constraining the widespread use and application of cold plasma in plants. This study aimed to explore the effects of low-vacuum helium cold plasma pre-treatments on the rooting capacity and root growth of zoysiagrass stolon cuttings as well as the preliminary regulatory mechanisms involved. The present study provides inspiration and support for new applications of cold plasma in plant growth and development.

2. Materials and methods

2.1. Cold plasma device

The cold plasma device is described in our previous article [9]. Radio frequency discharge plasma was used as a plasma source at a pressure of 150 Pa in this study. The cold plasma device consists mainly of three parts: a vacuum pump, a cold plasma generator, and a transmission device (figure 1). The volume of the discharge chamber is 4320 cm^3, and the distance between the two electrode plates is approximately 3 cm. In this study, the plasma frequency used was 13.56 MHz, the working gas was a mixture of helium and air, the electron temperature T_e^{high} was 3.5 eV, and the electron density n_e was approximately $10^{12}\ \text{cm}^{-3}$. The zoysiagrass stolon cuttings were treated with cold plasma as they passed through the cavity between the two plates.

2.2. Plant materials and cold plasma treatment conditions

Zoysiagrass ((*Z. japonica* × *Z. tenuifolia*) × *Z. sinica* var. *nipponica* cv. Z415-3, which is currently used commercially) stolon cuttings were 5–6 cm in length and 3.0 mm in diameter, and consisted of the first two nodes. The Z415-3 stolon cuttings were collected from the Hushu experimental station of the Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences (118 °46′ E, 32 °03′ N).

The stolon cuttings were exposed to 0, 100, 200, 300 and 400 W of cold plasma treatment for 15 s immediately after collection. Control (CK) stolon cuttings were also subjected to the same vacuum and helium flux as the treated stolon cuttings but did not receive any cold plasma treatment. The physiochemical properties of the stolon cuttings were then immediately determined, after which they were transplanted into a nutrient solution.

2.3. Experimental design

A hydroponic nutrient experiment was conducted at the Institute of Botany, Jiangsu Province and the Chinese Academy of Sciences, Nanjing, China (118 °46′ E, 32 °03′ N), from June 2016 to September 2017. The cold plasma-treated and CK stolon cuttings were planted in polyethylene plastic buckets (17 cm in diameter and 20 cm in height) that contained 2.5 l of half-strength Hoagland nutrient solution [23]. Each bucket was covered with a polyethylene plate that had 14 evenly spaced holes, and one stolon cutting was fixed into each hole. The buckets were incubated in a greenhouse under natural light wherein the temperature and humidity varied from 28 °C to 35 °C and from 60% to 80%, respectively. The nutrient solution was changed every four days. The experiments were conducted in a completely randomized design and were replicated three times, with one polyethylene plate per replication. There were 14 stolon cuttings per replicate, 42 stolon cuttings per treatment, and 210 stolon cuttings in the experiment.

2.4. Determination of rooting percentage

The number of rooted stolon cuttings was recorded daily for 8 d (No. stolon cuttings rooted). The rooting percentage was calculated as follows:

Rooting percentage (%) = Number of stolon cuttings rooted after 8 d/total number of stolon cuttings × 100%

2.5. Determination of root growth parameters

Nine stolon cuttings per treatment (three plants per polyethylene plate) were collected and washed with distilled water at one, two and three weeks after planting to analyze the root growth parameters. The total root length, total root surface area, total root volume and average root diameter were measured using a root-scanning apparatus (STD 4800, Epson,

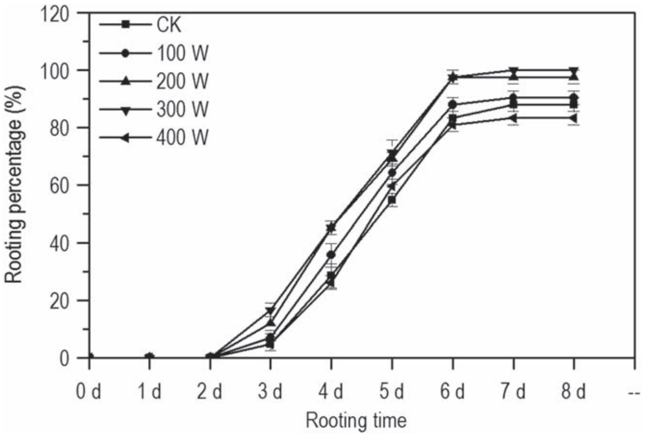

Figure 2. Influence of cold plasma treatments on the rooting percentage of Z415-3 stolon cuttings. The error bars represent the SEs.

Canada) and analyzed by WinRHIZO version 5.0 (Regent Instruments, Canada). The dry weight was determined after the roots were oven dried at 80 °C for 48 h to constant weight.

2.6. Determination of water uptake and relative conductivity

After the cold plasma treatment, the water uptake and relative conductivity of the treated and non-treated stolon cuttings were measured immediately. The water uptake of the stolon cuttings was assayed according to the methods of Turk and Tawaha [24]. A conductivity meter (model DDS-307, China) was used to measure the conductivity, and the relative conductivity was calculated according to the methods of Turk and Tawaha [24].

2.7. Statistical analysis

The data were presented as the means ± standard error (SEs). SPSS statistical software version 16.0 (SPSS, Inc., Chicago, IL, USA) and Origin 8.0 software (OriginLab Inc., Northampton, MA, USA) were used for statistical analyses, and the variance ($P < 0.05$) of the data was analyzed by one-way ANOVA (Duncan's test).

3. Results

3.1. Rooting percentage

The 200 and 300 W cold plasma treatments significantly improved the rooting percentage from 3 to 8 d after planting (figure 2). The final rooting percentage of the CK was 88.10%, which significantly increased by 10.82% and 13.51% in response to the 200 and 300 W treatments, respectively. The highest rooting percentage occurred via the 300 W treatment. However, the 100 and 400 W treatments had no significant influence on rooting percentage.

3.2. Root growth

The root growth was influenced by the cold plasma treatments (figure 3). Compared with that in response to the CK treatment, the total root length of the stolon cuttings in response to the 300 W treatment increased significantly by 37.28%, 35.28% and 26.81% at one, two and three weeks after planting, respectively (figure 3(a)). The 300 W treatment significantly increased the total root surface area at one and three weeks, which was 31.19% and 18.94% higher, respectively, than that of the CK (figure 3(b)). Moreover, the total root surface area markedly improved in response to the 100, 200 and 300 W treatments at two weeks; the total root surface area was 19.54%, 35.60% and 38.59% higher, respectively, than that in response to the CK. The 200 and 300 W treatments clearly increased the total root volume both at one week and three weeks, whereas the 300 W treatment significantly increased the total root volume at two weeks (figure 3(c)). With the exception of the 400 W treatment, all cold plasma treatments significantly increased the average root diameter at one or two weeks, and the 200 and 300 W treatments significantly increased the average root diameter at three weeks (figure 3(d)). The highest average root diameter was obtained in response to the 300 W treatment; compared with that in response to the CK treatment, the average root diameter in response to the 300 W treatment increased by 12.40%, 15.93% and 31.01%, respectively, after one, two and three weeks of planting. The dry weight of the roots significantly increased in response to the 300 W treatment at one week and increased in response to the 200 and 300 W treatments at two or three weeks, respectively (figure 3(e)). The maximum root dry weight was obtained in response to the 300 W treatment, and the values were 22.83%, 20.14% and 18.83% higher than those in response to the CK treatment, respectively, after one, two and three weeks of planting. With the exception of the average root diameter at two weeks, all root growth parameters were maximal in response to the 300 W treatment, whereas no pronounced differences in these parameters were observed between the 400 W treatment and the CK treatment during the entire rooting period.

3.3. Water uptake and relative conductivity

During the first 12 h, the water uptake rapidly increased, continued to increase towards its peak at 12 h and then slowly decreased, regardless of cold plasma pre-treatment (figure 4(a)). Both the 200 and 300 W treatments significantly improved the water uptake at 12 h (the values were 25.85% and 39.91% higher, respectively, than those in response to the CK treatment) and 24 h (36.24% and 62.24% higher, respectively, than those in response to the CK treatment). The 300 W treatment resulted in the best improvement in water uptake, whereas the 100 and 400 W treatments had no clear effects on water uptake. With the exception of the 100 W treatment, all the cold plasma treatments significantly increased the relative conductivity (figure 4(b)). Compared with that in response to the CK treatment, the relative conductivity of the stolon cuttings in response to the 200, 300 and

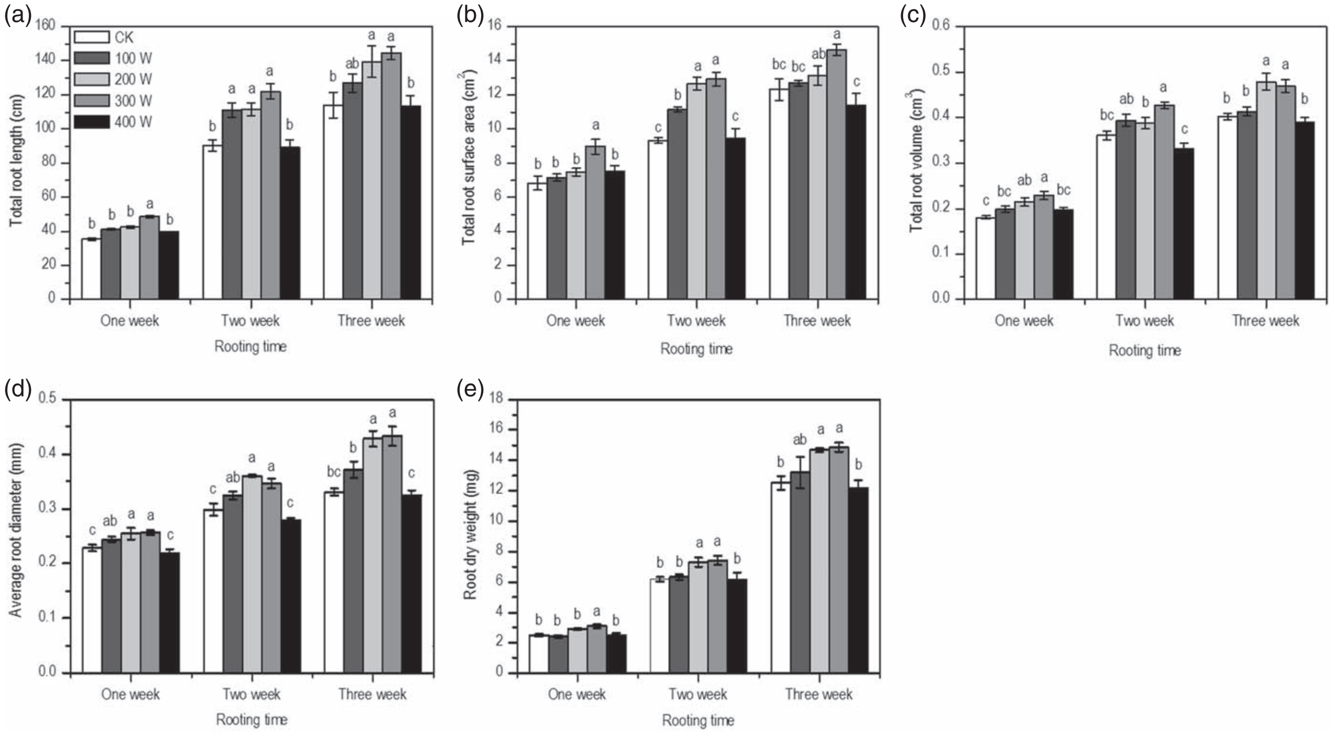

Figure 3. Influence of cold plasma treatments on the total root length (a), total root surface area (b), total root volume (c), average root diameter (d) and root dry weight (e) of Z415-3 stolon cuttings after one, two and three weeks of planting. The error bars represent the SEs. The different lowercase letters for the same rooting time indicate significant differences ($P < 0.05$ by Duncan's test) among all treatments.

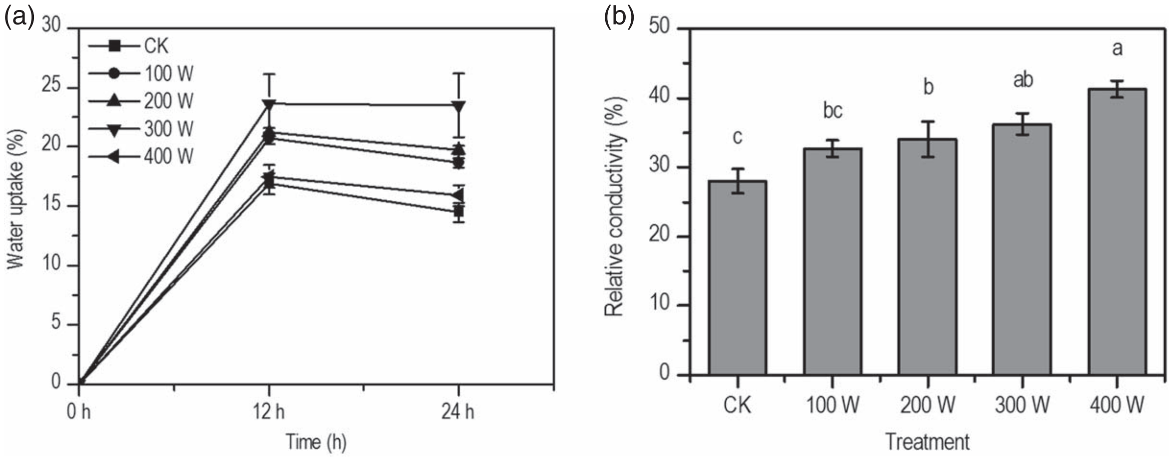

Figure 4. Influence of cold plasma treatments on the water uptake (a) and relative conductivity (b) of Z415-3 stolon cuttings. The error bars represent the SEs. The different lowercase letters indicate significant differences ($P < 0.05$ by Duncan's test) among all the treatments.

400 W treatments significantly increased by 21.59%, 29.18% and 47.26%, respectively.

4. Discussion

Studies have been conducted on the cold plasma pre-treatment of seeds to improve seed germination, plant growth (including root growth) and development. However, to date, the effects of cold plasma treatment on vegetative propagation, including rooting, have been unclear. This lack of knowledge greatly restricts the widespread use and application of cold plasma to plants. Zoysiagrass is an important warm-season turfgrass species that is widely used worldwide. Lower rooting capacity and slower root growth is a primary limiting factor for the establishment and production of this species [17]. Thus, in the present study, we studied the effects of cold plasma pre-treatment on the rooting percentage, root growth and physiochemical properties of zoysiagrass stolon cuttings.

Cold plasma treatment positively improved the rooting percentage (figure 2) and root growth of the zoysiagrass stolon cuttings (figure 5), and the 300 W treatment had the best promotive effect. However, the cold plasma treatments

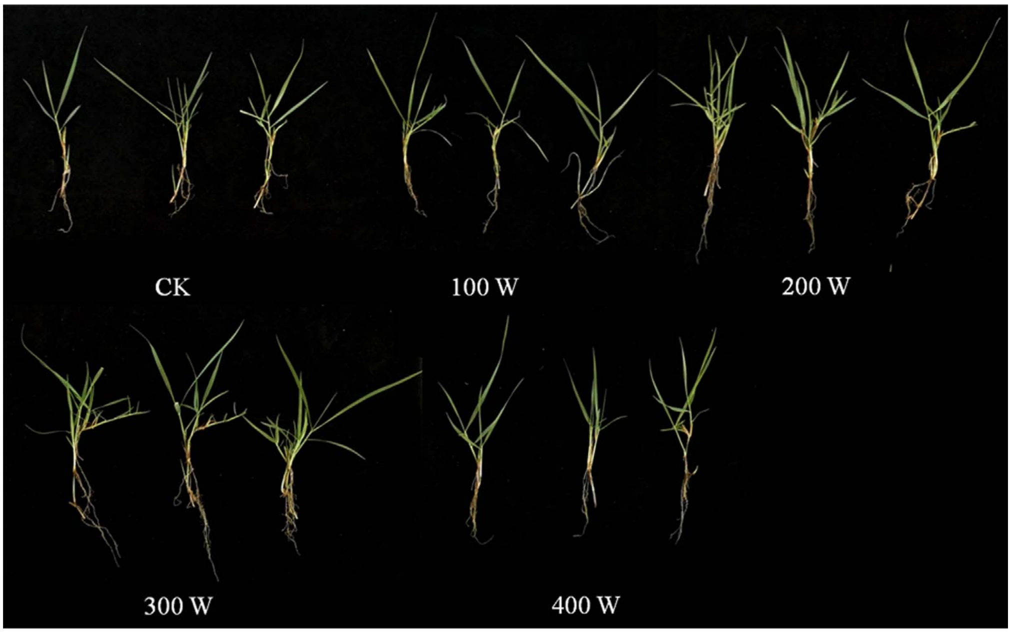

Figure 5. Influence of cold plasma treatments on the root growth of Z415-3 stolon cuttings.

with higher or lower power levels had no marked effects on the rooting ability or root growth of zoysiagrass stolon cuttings.

Water is critical for the successful rooting and root growth of stolon cuttings [25–27]. Increased water uptake capacity induced by cold plasma treatment might improve the rooting capacity of stolon cuttings [22, 28]. In the present study, the water uptake in zoysiagrass stolon cuttings clearly increased in response to cold plasma treatment (figure 4(a)). The difference in water uptake of stolon cuttings was induced by changes in membrane permeability [29]. The permeability of stolon cuttings can be reflected by their relative conductivity. The results of the present study showed that the relative conductivity of the zoysiagrass stolon cuttings markedly increased in response to cold plasma treatment (figure 4(b)), thus revealing that the cold plasma treatments strongly increased the permeability of the stolon cuttings. Since the permeability of stolon cuttings was improved by the cold plasma treatment, their ability to absorb water and nutrients relatively increased, which in turn might stimulate physiological metabolism related to rooting, leading to a higher rooting percentage and improved root growth. The combined interaction between the cold plasma-treated stolon cuttings and plasma-activated water uptake is unclear. Blockage at the bottom of the stem is the main internal cause affecting the water absorption of stolon cuttings [27]. The base of stolon cuttings is usually blocked by microorganisms and their metabolites [27]. Cold plasma has been reported to reduce the activity of these microorganisms effectively [3] and improve the permeability of seeds [5, 30]. Therefore, we speculated that cold plasma might improve the permeability of the base of stolon cuttings by reducing the activity of microorganisms, ultimately improving water uptake.

Plant roots are the functional organs for water and nutrient absorption. Previous studies have indicated that plasma pre-treatment of seeds improves root growth [8, 9, 13]. However, there have been no reports on the effects of cold plasma pre-treatment on the root growth of vegetative plant parts. In the present study, root morphological characteristics, including total root length, total root surface area, total root volume and average root diameter, were markedly improved by cold plasma treatment, which putatively contributed to increased root dry weight (figure 3). Plants with relatively great root length, surface area, volume and diameter have been associated with relatively high water and nutrient concentrations, which leads to robust growth and rapid turf establishment.

5. Conclusions

In summary, cold plasma treatment effectively improved both the rooting percentage and root growth of zoysiagrass stolon cuttings. The increase in rooting percentage and root growth may be attributed to the increased permeability and water uptake of the cuttings. Thus, cold plasma could be used in future vegetative propagation in economically important plants. Additional research is needed to explore the mechanisms underlying the effects of cold plasma treatment on the rooting and root growth of zoysiagrass stolon cuttings.

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