Improved Sprout Emergence of Garlic Cloves by Plasma Treatment

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ABSTRACT: High growth and yield of food crops are desired due to globally increased food demands. Highly reactive gaseous plasma treatment technologies have been recently shown to improve the growth of various types of seeds, and we attempted to study the influence of different treatment conditions on the growth of garlic cloves. Inductively coupled, low-pressure, radio frequency oxygen plasma was used for the treatment of garlic cloves. We studied the influence of plasma treatment conditions on garlic sprout emergence as well as on wettability, surface chemistry, and morphology of garlic skin (the protective leaf). Our findings show that at appropriate plasma treatment conditions, improved sprout emergence of garlic cloves is observed.

KEY WORDS: plasma treatment, garlic, sprout, emergence, growth, oxygen

I. INTRODUCTION

With increasing demands and various factors affecting production of food, an improvement in the germination and growth of food crops—as a mechanism for increasing yields—is highly desired. Agricultural sustainability will need to meet increasing food demands without producing harmful environmental effects. Garlic (Allium sativum L.) is an important agricultural crop, with world production totaling more than 24 million tons in 2013. In garlic production, high-yield and -quality crops are desired, but production is limited by region and climate. Cereals and other agriculturally important plants are propagated sexually, through seeds. In contrast, although fertile clones of garlic were indeed discovered in central Asia some three decades ago, the commercially cultivated varieties of garlic are sterile. Because they do not produce seeds, they must be propagated vegetatively, that is, individual cloves must be planted in soil, where they germinate and produce new plants. Although improvement of crops such as cereal, and even other alliums, is possible by conventional or innovative breeding techniques, these methods are not applicable to agriculturally produced garlic. Therefore, alternative methods of crop improvement must be considered to improve germination and growth (without the need for genetic engineering) and to propagate established or autochthonous cultivars instead.
Some techniques influencing garlic germination have been studied, but the available literature is currently very limited. Considering chemical treatments, Kuroda et al. applied erythritol, a sugar alcohol, to garlic cloves, decreasing germination time and enhancing growth at low concentrations. Rahman soaked cloves in gibberellic acid, a plant hormone and growth regulator, before planting and observed an influence on germination percentage, but with irregular results. Although these approaches are ecologically less applicable to commercial agriculture, they may provide insights into dormancy and germination mechanisms in a laboratory setting.

Regarding physical treatment, the majority of available data on garlic cloves describe exposure to gamma radiation. Irradiation, however, was mostly performed to inhibit germination to prevent spoiling during storage. The effects differed with dose and clove state during irradiation (dormancy or postdormancy), and most treatments were carried out at high-radiation doses. Currently, no available data point to germination improvement by gamma irradiation; low irradiation dose did not affect sprouting or accelerated germination, but it reduced the number of sprouting calluses, as shown in a study that was performed on tissue culture in vitro that aimed for mutation induction. The postulated involvement of plant growth regulators suggests optimization possibilities, but the treatment currently does not seem feasible in regard to cost and safety.

The only other investigated physical treatment was temperature. Freshly harvested garlic cloves are dormant and will not readily germinate, but temperature is one of the factors affecting this dormancy. To achieve sprouting, vigor of growth, and bulbing, seed cloves must be exposed to low temperatures for an optimal amount of time. This normally occurs after planting in the field, but cloves can also be exposed to low temperatures during storage. Thermopriming of seeds may be effective in improving seed germination, and the effect of garlic clove storage temperature was also studied in several recent publications. However, contradictory results were reported, including improved clove yields of spring-planted garlic after chilling at 4°C and faster development but lower yields, lower bulb size, or weight after conditioning at 5°C or 10°C. The rate of bulbing with a single clove also increased and, in general, may occur at very rapid bulbing, but this is not desired. In addition, the required amounts of exposure to low temperatures are also quite long, and an alternative method that could avoid prolonged storage times is favored.

Since the emergence of plasma agriculture, many reports have detailed the impact of plasma treatment on the germination and growth of agricultural seeds. In addition to variously defined germination and growth parameters, several sources also considered effects on seed surface morphology and chemistry. A review of nonthermal plasma treatment of agricultural seeds has recently been provided by Randeniya and de Groot, who cited several instances of seed plasma treatment wherein germination or growth improvement was achieved. In contrast, scant literature is available on sprout emergence of garlic cloves, particularly on sprouting, growth, and yield improvement. To the best of our knowledge, plasma treatment of garlic cloves with the intent of improving germination has not yet been studied.
Plasma treatment has several advantages over other treatment techniques described above. As opposed to different kinds of priming, it does not expose the biological material to moisture and therefore does not require a drying step before storage that can counteract the beneficial effects of the treatment. It can also be performed rather rapidly and is an environmentally friendly process, because it does not produce chemical waste.

In the present work, we used a low-pressure, inductively coupled, radio frequency (RF) oxygen plasma to treat garlic seed cloves. Inductively coupled plasma exists in two modes: the so-called E mode, characterized by lower electron densities and lower luminosity, and an H mode, characterized by higher electron densities and higher luminosity. In the system, an electrostatic field is present due to potential differences between the induction coil and the ground surfaces of the system. At lower-input powers, in E mode, this electrostatic field is larger than the induced field and, thus, the plasma discharge is primarily formed as a consequence of capacitive coupling. In H mode, the induced electric field is larger and is the primary factor sustaining the discharge. At the same time, the electrostatic field, and thereby capacitive coupling, remains present. Besides being exposed to H-mode afterglow plasma, garlic cloves are also exposed to this additional capacitively coupled plasma, known as stray capacitance or parasitic capacitance.

The aim of the present study was the application of gaseous plasma to improve germination of garlic and to characterize the chemical and morphological changes occurring at the garlic skin surface. To study the influence of plasma on the germination of garlic cloves, cloves were exposed to highly reactive oxygen plasma for different treatment durations. The influence of plasma was studied by measuring germination as well as changes in surface wettability, surface morphology, and chemistry of the garlic clove skin.

II. MATERIALS AND METHODS

A. Garlic Clove Material

Garlic cloves of a Slovenian autochthonous cultivar (*Ptujski jesenski*) were used in the experiments. Garlic heads were picked apart and the outer layers of the skin removed. The cloves themselves were left unpeeled, so that the final layer of skin (the protective leaf) remained intact. For the sake of clarity, this protective leaf is referred to in this article as “garlic skin.” The mass of each individual clove was recorded before plasma treatment.

B. Plasma Setup

As mentioned above, the garlic cloves were treated using a low-pressure, inductively coupled, RF oxygen gaseous plasma. The plasma system consisted of a borosilicate discharge tube. A copper coil was wrapped around the discharge tube, and an RF generator operating at 13.56 MHz was coupled to the coil via a matching network. On the genera-
tor, nominal power was adjustable, and the reflected power was recorded to indicate the effectiveness of the power coupling. The discharge tube was continuously pumped using a two-stage oil rotary vacuum pump at a nominal pumping speed of 80 m$^3$/h to sustain the desired low pressure. Pure oxygen gas was introduced at the opposite end of the tube by a mass flow controller (AERA PC7700, Advanced Energy, Fort Collins, CO, USA), calibrated for oxygen at 20°C. Commercially available oxygen of 99.99% purity was used. Pressure was monitored using a baratron absolute-pressure gauge (MKS Instruments, Andover, MA, USA; see Fig. 1).

During plasma treatment, the coil was positioned at a distance of 225 mm from the joint of the tube and the metal T piece. Cloves were positioned at a distance of 300 mm from the side of the coil (plasma afterglow), closer to the pumped end of the discharge tube, as illustrated in Fig. 1. The plasma was ignited at a pressure of 30 Pa, with a forward power of 300 W and a reflected power of 18 W, resulting in H-mode plasma. This type of plasma is mostly spatially enclosed; however, stray capacitance remains present throughout the discharge tube.

![Schematic of the plasma system used in the experiments](image)

**FIG. 1:** Schematic of the plasma system used in the experiments

### C. Plasma Treatment of Cloves

In a preliminary test at the specified conditions, a treatment time of 60 s was established as the maximum treatment time before charring of the protective leaf occurred. Thus, treatment times up to 60 s, in intervals of 15 s, were chosen for the experiment. Cloves were placed on a microscope slide inside the discharge tube and treated consecutively, one by one. During treatment, the tops of the cloves were oriented toward the gas source. Untreated cloves were used as the control. The effect of only low pressure and gas flow, without the use of plasma, was also evaluated. These results are not included, because no influence on garlic germination was observed.

*Plasma Medicine*
D. Sprout Emergence

After a storage period of 3 mo, cloves were treated by plasma. For each plasma treatment time, we used four cloves that were uniform in size. To sprout the cloves, each individual clove was wrapped in an approximately 4 cm × 5 cm piece of paper towel, placed on a petri dish and moistened with 1.5 mL of tap water. The incubation begins with placing the clove in wet paper towel in a petri dish (t = 0). Garlic cloves were incubated at room temperature under alternating light conditions (light, dark). After 165 h, the paper towels were replaced to avoid mold, and 1 mL of water was applied to each clove.

The length of the sprout was recorded in varying time intervals, up to a total of 310 h after the start of sprouting. Sprouts were measured from their lowest point of emergence from the garlic storage leaf up to the tip of the sprout (Fig. 2). At earlier measurements, short sprout lengths (before the sprout protruded through the protective leaf) were estimated. Some cloves decayed at certain points during the experiment and were ignored in calculations from that point on. This occurred for two untreated cloves (one at 190 h and one at 238 h) and for one clove treated for 45 s (at 214 h). The majority of cloves decayed at 310 h.

E. Water Contact Angle

The wettability of reasonably flat garlic clove skin surfaces was evaluated by performing a contact angle analysis of a 3-μL droplet of tap water on the surface. Static contact angle by the sessile drop method was evaluated via a surface energy evaluation system (Advex Instruments, Brno, Czech Republic), equipped with a charge-coupled device.
camera. Measurements were taken at room temperature (22°C) and at constant relative humidity (60%). Aging was recorded by measuring the contact angle on treated cloves in time intervals ranging from 1 to 72 h, as well as 1 wk after treatment. Between measurements, the cloves were stored at room temperature (22°C) and at constant relative humidity (60%).

F. X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) analysis was performed to obtain elemental composition of the clove surface. Using a scalpel, ~4 × 4–mm pieces of garlic skin were removed from treated and untreated cloves. The samples were mounted onto an XPS sample holder, and their surfaces were excited by X-ray radiation from a monochromatic Al source at photon energy of 1486.6 eV. High-energy resolution spectra were acquired with an energy analyzer operating at a resolution of ~0.5 eV and a pass energy of 29 eV. Spectra were calibrated using a C1s peak at 284.8 eV. Three different XPS measurements were performed on each sample, and the average chemical composition was calculated.

G. Scanning Electron Microscopy

For scanning electron microscopy (SEM) analysis, we used a scalpel to remove ~4 × 4–mm portions of garlic skin from treated and untreated cloves. After drying in a vacuum chamber, the samples were attached onto aluminum stubs using conductive carbon tape and then coated with a thin layer of gold using a Balzers SCD 050 sputter coater (Bal-Tec, Balzers, Liechtenstein). The SEM images were obtained using a Jeol JSM-7600F Schottky field emission scanning electron microscope (JEOL, Ltd., Tokyo, Japan).

III. RESULTS AND DISCUSSION

A. Sprout Emergence

Results of the sprout emergence of untreated and plasma-treated garlic cloves are presented in Figure 3. It can be observed that in the first hours of the process (up to 100 h), the sprout length was highest for the 30-s plasma treatment, followed by the 45-s treated sample and untreated sample. However, with longer times (after 150 h) of incubation, a significant increase in sprout length can be seen for the 45-s treated sample. Results show that treatment times up to 45 s improve sprout length, whereas longer treatment (60 s) already results in diminished sprout length compared to the control.

The present study shows that the optimal treatment time at specified conditions is 45 s, resulting in an ∼300% increase in the sprout-length parameter. Improvement is also observed for shorter treatment times (15 and 30 s). The effect is particularly pronounced after germination times of 75 h and above. An increase in sprout length after plasma treatment was often found in seeds,25–30 but has not yet been recorded in garlic (to the best of our knowledge). Other studies report that sprout-length increase also depends on the feed
B. Changes in Surface Wettability

Other research has proposed several potential mechanisms (that appear to be interconnected) for germination and growth improvement of seeds. Improvement of surface hydrophilicity is a key concept in these studies of seed-germination improvement, showing that more efficient direct exposure of seed surface to water can improve the germination process. To the best of our knowledge, data regarding imbibition of postdormant garlic cloves, or its relation to germination, are currently unavailable. However, for seeds, imbibition is a well-established concept and the first step in the germination process.33 Increased hydrophilicity of the surface is thus desired to enhance seed hydration.

Changes in wettability of garlic skin surface provide initial information about plasma-induced changes on the surface of garlic cloves. Untreated garlic skin has an increased hydrophobic character, with a water contact angle (WCA) of $\sim 105^\circ$, and even after only 15 s of plasma treatment, the surface becomes more hydrophilic, with a WCA of $\sim 60^\circ$ (Fig. 4). The values observed at 15, 30, and 60 s are comparable, and the WCA value after 45 s of treatment is the lowest, at $\sim 45^\circ$. Interestingly, this correlates well with the germination results: garlic treated for 45 s exhibits the most efficient germination improvement. In the available literature, connections of improved wettability to plasma...
treatment and germination speed were observed for lentil, bean, and wheat seeds.\textsuperscript{19,34} After plasma treatment, Li et al.\textsuperscript{26} and Dobrin et al.\textsuperscript{27} also noted increased wettability in treated soybean and wheat seeds, respectively, along with improvement in some growth parameters.

A well-known phenomenon of plasma-treated surfaces is so-called hydrophobic recovery (aging effect) of the surface; thus, we analyzed the wettability of plasma-treated garlic cloves after aging at room temperature for different time intervals. Hydrophobic recovery was indeed observed for all plasma-treated garlic skin surfaces, even after only 1 h of aging. For the 45-s treated surface, WCA increased from $\sim 45^\circ$ to $\sim 74^\circ$. Similar wettability was also observed for other treatment conditions. After longer storage, no significant changes in wettability were observed and all plasma-treated surfaces reached a stable value of $\sim 85^\circ$ after 1 wk of aging. This value is still lower than that of untreated garlic skin and could have a role in the growth mechanism of garlic cloves in the field. It should be noted that similarly to seeds,\textsuperscript{33} garlic surface is not flat and uniform; thus, quantitative determination of WCA is difficult and subject to high standard deviations. Imperfections in the clove surface, differences among individual cloves, and general variability of biological material can all affect individual measurements that would be reflected in the standard deviation. Nonetheless, a qualitative determination is generally possible and gives some indication of changes induced at the surface.

Conversely to our results, Guimarães et al.\textsuperscript{35} noted no hydrophobic recovery in leucaena seeds within 24 h of plasma treatment with a dielectric barrier discharge helium
plasma jet, and Bormashenko et al.\textsuperscript{19} did not observe hydrophobic recovery within 1 mo of treating wheat seeds with inductively coupled low-pressure air plasma.\textsuperscript{19} The difference may be due to variance in chemical composition of the respective surfaces or to the plasma setup and conditions used. The observed increase in hydrophilicity of garlic and other similar materials by highly reactive oxygen plasma can be explained by the alternations in surface chemistry and morphology that can occur during treatment as reported below.

\section*{C. Changes in Surface Chemistry}

Results of the elemental composition analysis obtained from XPS for untreated and plasma-treated garlic skin surfaces are presented in Table 1. A decrease in carbon content and an increase in oxygen content of the garlic skin were observed after 15 s of treatment. Small amounts of other elements such as Si, Ca, and K were also detected on the plasma-modified surfaces, which could be explained by an etching effect on the garlic skin by the plasma species. No significant changes in the chemical composition of garlic skin were observed for 15-, 30-, and 45-s treatment times, as seen in Table 1, whereas a significant increase in oxygen-containing functional groups was detected after 60 s of plasma treatment. Moreover, at this treatment time, an increase in Ca and K was also observed, which could be correlated with higher etching of organic material. Moreover, differences between high-resolution XPS carbon C 1s spectra of the samples were also detected due to different chemical bonds on the garlic skin surface (Fig. 5). In cases of untreated garlic skin, the main peak is observed at the binding energy of 284.8 eV, corresponding to C–C and C–H bonds, and two small subpeaks appear at 286.4 and 288.9 eV, corresponding to C–O and O–C=O components. For case of 15-, 30-, and 45-s plasma-treated surfaces, an increase in subpeaks corresponding to C–O and O–C=O components was observed, and a subpeak at 287.8 eV, corresponding to the O–C–O component, was also detected for the 45-s treated surface. A significant difference in the carbon C 1s peak was observed for the 60-s treated surfaces (Fig. 5d). In this case, an increase in peaks corresponding to C–O, O–C–O, and O=C–O components was observed. The C 1s spectra in this case have a similar shape to those in cellulose materials, which are also one of the building blocks of deeper layers of garlic skin. At this treatment time,

\begin{table}[h]
\centering
\caption{Elemental composition of the garlic skin surface determined from XPS analysis on untreated and plasma-treated surfaces}
\begin{tabular}{cccccccc}
\hline
Elemental Composition (%) & C & O & N & Si & Ca & K \\
\hline
Treatment time (s) & 0 & 15 & 30 & 45 & 60 \\
\hline
C & 96.9 & 75.8 & 77.4 & 77.9 & 56.0 \\
O & 3.1 & 21.4 & 20.4 & 19.4 & 39.7 \\
N & - & 0.7 & 0.8 & 2.3 & 1.9 \\
Si & - & 1.5 & 0.9 & 0.5 & 0.6 \\
Ca & - & 0.6 & 0.5 & - & 1.2 \\
K & - & - & - & - & 0.6 \\
\hline
\end{tabular}
\end{table}
reduced sprout emergence was also observed and to some extent may be correlated with changes in surface chemistry—not only due to the increase in oxygen-containing functional groups (that could be the result of intense etching and removal of epicuticular wax) but also to functionalization with oxygen species that influence the biochemical processes that stimulate sprout emergence.

As with polymer materials, it is possible to use plasma treatment to introduce new functional groups to the organic materials of plant surfaces, thereby increasing the surface energy to cause hydrophilization. Bormashenko et al. have linked improved wetting of wheat seeds directly to functionalization with air plasma, namely, by introduction of oxygen-containing functional groups. This was confirmed by time-of-flight-secondary ion mass spectrometry analyses that showed 2.5 to 3 times more intense mass peaks of oxygen for plasma-treated seeds. Filatova et al. used air plasma treatment of seeds and suggested the process of chemical etching of seed surface by surface oxidation. On the other hand, Kitazaki et al. worked with O$_2$ plasma, and from optical emission spectra, suggested exposure to oxygen radicals.

Oxygen plasma is known for its ability to functionalize organic materials via oxygen radicals, increasing hydrophilicity by the introduction of oxygen-containing functional groups, which was also observed in our study. The newly formed oxygen functional groups on the surface do not only influence surface wettability but could, as such, play an important part in the stimulation of biochemical processes that influence germination.

**FIG. 5:** High-energy-resolution XPS spectra C 1s obtained from (a) untreated, (b) 15-, (c) 45-, and (d) 60-s plasma-treated garlic skin surfaces
D. Changes in Surface Morphology

Morphology of untreated and plasma-treated garlic skin surface was studied using SEM. Significant changes in surface morphology were observed immediately after plasma treatment (15 s). The untreated surface was relatively smooth with no special surface topography (Fig. 6a), whereas the plasma-treated surface was modified (Fig. 6b). Weak abrasion of the surface was observed after 15 s of plasma treatment. Interestingly, no significant changes in surface morphology compared to the 15-s treatment were detected after longer treatment times, even in the case of 60-s treatment (images not shown).

Etching of the surface by plasma species is another possible effect of plasma treatment and could contribute to increased wetting and water uptake by modification of surface roughness or improvement of skin permeability. It is suggested that plasma treatment of seeds results in the erosion of a waxy layer at the seed surface. Because the protective leaf of a garlic clove exhibits a cuticle on its outer side, a waxy layer is also expected on the surface of the unpeeled clove. Removal of such a hydrophobic layer by etching will thus facilitate wetting and water absorption.

FIG. 6: SEM images of garlic skin surface for (a) untreated and (b) 15-s plasma-treated garlic (30,000× magnification)
IV. CONCLUSIONS

Results of our study show that by low-pressure oxygen plasma treatment, increased sprout emergence of garlic cloves was achieved. All plasma-treated surfaces exhibited improved wettability of garlic skin. Changes in surface morphology of garlic skin were also detected; however, no significantly altered surface morphology was observed between 15 and 60 s of plasma treatment. Increases in oxygen-containing functional groups on the surface of garlic skin were observed after 15 s of treatment that can, to some point, be correlated with improved sprout emergence. Moreover, after 60 s of treatment, a prominent increase in oxygen functional groups on the surface was observed, whereas sprout emergence at this point was significantly reduced. It appears that surface functionalization of garlic skin could play an important part in stimulation of biochemical processes and influence the growth process in garlic cloves.

REFERENCES

35. Guimarães IP, Alves C, Jr., Torres SB, Vitoriano JO, Dantas NBL, Diogenes FEP. Double barrier di-


