Cold plasma interactions with plants: Morphing and movements of Venus flytrap and *Mimosa pudica* induced by argon plasma jet

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**Abstract**

Low temperature (cold) plasma finds an increasing number of applications in biology, medicine and agriculture. In this paper, we report a new effect of plasma induced morphing and movements of Venus flytrap and *Mimosa pudica*. We have experimentally observed plasma activation of sensitive plant movements and morphing structures in these plants similar to stimulation of their mechanosensors in vivo. Application of an atmospheric pressure argon plasma jet to the inside or outside of a lobe, midrib, or cilia in *Dionaea muscipula* Ellis induces trap closing. Treatment of *Mimosa pudica* by plasma induces movements of pinnules and petioles similar to the effects of mechanical stimulation. We have conducted control experiments and simulations to illustrate that gas flow and UV radiation associated with plasma are not the primary reasons for the observed effects. Reactive oxygen and nitrogen species (RONS) produced by cold plasma in atmospheric air appear to be the primary reason of plasma-induced activation of phytoactuators in plants. Some of these RONS are known to be signaling molecules, which control plants’ developmental processes. Understanding these mechanisms could promote plasma-based technology for plant developmental control and future use for plant protection from pathogens. Our work offers new insight into mechanisms which trigger plant morphing and movement.

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1. Introduction

The universe is composed of plasma - the most abundant yet least understood state of matter. On Earth, plasma is naturally produced in the form of lightning and the aurora borealis. Since the beginning of the 20th century, the effects of aurora borealis on plants and trees have been associated with ions generated near the Earth’s surface by atmospheric electricity. The associated research was termed “Electroculture” [1,2].

In physics and chemistry, plasma denotes a quasi-neutral mixture of electrons, ions and neutral species formed during gas ionization by fast free electrons. Due to the large difference of electron and atom mass, electron mean energy (temperature) may exceed gas temperature by two orders of magnitude when electricity passes through a gas. Low temperature (cold) plasma provides a non-equilibrium catalytic environment for a variety of chemical reactions at ambient gas temperatures [3]. In biology and medicine, plasma has a very different meaning as the water-like liquid component of blood. In the present paper, the term plasma refers to the fourth state of matter, i.e. a partially-ionized quasi-neutral mixture of electrons, ions, and neutral species.

In recent years, cold plasma has found an increasing number of applications in biology, medicine and agriculture, particularly for the treatment of crops, seeds, water and soil [4]. Experimental studies have demonstrated plasma-assisted decontamination of seeds, enhancement of seed germination, growth enhancement of plants, and reduction of microbial load in food [5,6]. The majority of the reported plasma treatment used low-pressure radio frequency systems, which require vacuum equipment [7,8]. Recently, atmospheric-pressure cold plasmas have been produced using plasma jets, dielectric barrier discharges (DBD) and corona discharges. Such plasmas are actively being studied for applications in medicine and biotechnology due to their ability to induce desirable biochemical responses in living organisms, plants and seeds [9–12]. Plasma treatment induced desirable changes of developmental and physiological processes in plants, improving seed resistance to stress and diseases, modifying seed coat structures, increasing the permeability of seed coats, and stimulating seed germination and seedling growth for several types of commercially significant food plants for human and animal consumption, such as wheat, barley, tomato, soybean, and *Arabidopsis thaliana*. The observed beneficial effects of plasma

Abbreviations: RONS, reactive oxygen and nitrogen species; ROS, reactive oxygen species.

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arise from the cocktail of reactive neutral species, charged species (electrons, ions), electric fields, and ultraviolet radiation produced in the discharge at ambient pressure and room temperature [13].

It is believed that reactive oxygen species (ROS) and reactive nitrogen species (RNS) created by plasma are mainly responsible for its beneficial effects in biology, medicine, agriculture, and food treatment [14, 15]. It is known that ROS participate in plant developmental processes by acting as signaling molecules for cell proliferation and differentiation, programmed cell death, seed germination, gravitropism, root hair growth, senescence, and pollen tube development, etc. However, the specific mechanisms responsible for the regulatory action of ROS at various plant developmental stages remain unknown [16]. While RONS are required for many important signaling reactions in plants, they are also toxic by-products of aerobic metabolism. As cold plasma becomes an attractive method for protection from viruses, fungi and bacteria in biology, medicine, food processing, and agriculture, it may also have side effects due to undesirable interactions of RONS with ion channels, membrane transport and bio-tissue.

Many plants can change shape and dimensions under external perturbations. This morphing behaviour can involve micro, macro, structural, and fluidic processes. The rapid closure of the carnivorous plant *Dionaea muscipula* Ellis (Venus flytrap) has been widely investigated [17–22]. The Venus flytrap can be closed by mechanical stimulation of trigger hairs [23] at the inner side of a lobe or by an electrical stimulation between a midrib (+) and a lobe (−) [24,25] causing the lobe to change its shape from convex to concave.

Another plant with morphing behaviour is the *Mimosa pudica*. Volkov et al. have previously investigated the morphing structures in *M. pudica* induced by electrical or mechanical stimulation [26–29].

In this paper, we report the first observations of cold plasma activation of the Venus flytrap and *Mimosa pudica* morphing structures using an argon plasma jet.

2. Materials and methods

2.1. Plant material

Fifty bulbs of *Dionaea muscipula* (Venus flytrap) were purchased for this experimental work from Fly-Trap Farm (Supply, North Carolina). The soil was treated with distilled water. The seeds of *Mimosa pudica* L. were soaked in warm water (30 °C) for 48 h. After growing for two weeks, the seedlings were transplanted into pots and placed inside a plant growing chamber. Two-three-month-old *Mimosa pudica* plants were used for the experiments. All plants were grown in well drained pots at 22 °C with a 12:12 h light: dark photoperiod. The plants were watered every day. Humidity averaged 45–50%. Irradiance was 700 μE/m²s. All experiments were performed on healthy adult specimens.

2.2. Chemicals

Hydrogen peroxide solution was purchased from Sigma-Aldrich (USA).

2.3. Experimental apparatus

The plasma source consisted of a nested pair of quartz tubes with a central tungsten rod and an outer steel ring (Schematic 1). The rod and ring served as the powered and grounded electrodes respectively. The outer tube (3 mm ID, 6 mm OD) was open on both ends and one end was held in the perpendicular leg of a plastic compression Tee fitting. The inner tube (1 mm ID, 2 mm OD) had a closed end and was inserted into the outer tube through a drilled hole in the Tee fitting and seal with epoxy. The 1 mm diameter tungsten rod was then placed inside the inner tube to rest against the seal end. The grounded ring electrode was placed near the end of the outer tube. The source design prevented arcing that can occur if the powered rod were in direct contact with plasma. Argon gas was flown at a rate 1.55 L/min between the two tubes. The argon was ionized by the strong electric fields between the electrodes, and formed a plasma jet approximately 2 cm long measured from the exit of the outer tube. The plasma was driven with a high voltage pulsed DC circuit consisting of a Matsusada AU-10P60 10 kV DC power supply, a IXYS PVX-4110 pulsed generator, and a SRS DG-645 delay generator. The system was operated with 8 kV pulse amplitude, 8 kHz pulse frequency, 500 ns pulse width, and a ~70 ns rise time. An electrical system for monitoring the voltage and current waveform contained a PR-55 high voltage probe and a Pearson 411 current monitor connected to a Tektronix MDO3024 oscilloscope. The voltage had a square pulse shape with a slight sloping during the rising edge. The current trace had two distinct current pulses at the rising and falling fronts of the voltage with damping oscillations after the main current peaks due to both displacement and conduction currents [30].

2.4. Video recording

Digital video recorders, Sony DCR-HC36 and HD AVCHD, were used to monitor the Venus flytraps and to collect digital images, which were analysed frame by frame using Sony Vegas 13 software. All experimental results were reproduced at least 14 times using different plants.

2.5. Software

Simulation results were performed using commercial multi-physics software CFD-ACE+. Software SigmaPlot 12 (Systat Software, Inc.) was used for statistical analysis of experimental data.

3. Results

3.1. Venus flytrap

The Venus flytrap can be closed by placing the plume of the argon plasma jet near the internal or external sides of the lobes, midrib, or cilia at about 2 cm distance from the plant surface (Fig. 1). However, application of the argon plasma jet to the lower leaf of the Venus flytrap for 40 s does not close the trap. Pure gas flow, without plasma, has no closing effects on the plant. All traps closed by the plasma were completely open again two or three days after the plasma treatment. Thus, cold plasma did not damage the plants. The same trap-closing experiments by plasma exposure were reproduced a second time with the same plants within a week. Fig. 1 illustrates the dynamics of trap closure induced by application of the plasma jet to an internal side of a lobe (Fig. 1a, and curve 1 on Fig.
1b) and to an external side of the lobe (curve 2 on Fig. 1b). The dynamics of trap closure by the plasma treatment of the lobe are very similar to the dynamics of the trap closure by mechanical simulation of the sensitive trigger hairs or by injection of a 14 μC charge between a midrib and a lobe [24]. Curve 3 on Fig. 1b shows the dynamics of the trap closure induced by application of a plasma jet to the cilia. There is about a 4 s delay between the beginning of plasma treatment of the cilia and the closure of the trap. Comparatively, mechanical or electrical stimulation of the cilia did not close the trap.

3.2. Mimosa pudica

*Mimosa pudica* Linn. is a thigmonastic or seismonastic plant in which the leaves close and the petiole hangs down in response to certain stressors. The plant contains long slender branches called petioles, which can fall due to mechanical stimuli. The petioles contain smaller pinnae, arranged on the midrib of the pinna. The pinnules are the smallest leaflets while the entire leaf contains the petioles, pinnae, and pinnules. A pulvinus is a joint-like thickening at the base of a plant leaf or leaflet that facilitates thigmonastic movements. Primary, secondary, and tertiary pulvini are responsible for the movement of the petiole, pinna, and leaflets, respectively (Fig. 2).

Our treatment of *Mimosa pudica* pinnules or pulvini by the cold plasma jet induced movements of pinnules and rachis (Fig. 3a), similar to mechanical (Fig. 3b) or electrical stimulation [26–29]. Plasma stimulation of a pinnule caused closure of all pinnules at pinnae.

We have directly tested that the same neutral argon gas jet in the absence of plasma does not produce plant activation. We have confirmed that placing plants next to the discharge tube, where UV and visible radiation has maximal values, has no effect on the plants. Also, charged species (electrons and ions) may not be the cause because the plant activation is produced by the “tail” of the plume (see Schematic 1) where the density of charged particles decays substantially.

We have performed simulations of argon jet in air using Reynolds averaged gas mixing model. Fig. 4 illustrates that the jet velocity does not exceed 5 m/s (Fig. 4b) and the jet extends for about 2 cm into air, which is consistent with experimental observations of the plasma
plume emanating from the tube (Fig. 4a). Although the plasma plume appears steady (Fig. 4a), it consists of guided ionization waves traveling along the argon jet channel at speeds orders of magnitude faster than the gas flow velocity. Detailed simulations of gas breakdown, plasma formation, propagation of the ionization waves, and electron-driven plasma chemistry in the argon-air mixing layer are beyond the scope of this paper. In particular plasma chemistry is rather complex including a multitude of species and dozens of reactions [31]. Hence simulations of plasma jets have been performed so far only with simplified chemistries [32,33] or with global plasma models [34].

The most likely species responsible for the observed effects of cold plasma on plants are ROS, including peroxides (H₂O₂). Hence, we have tested whether hydrogen peroxide can indeed induce closing of the Venus flytrap in the absence of plasma. Two 10 μL drops of H₂O₂ were placed on the midrib of the trap. After a few seconds, about 80% of traps were closed. Higher concentration of H₂O₂ produced more closing traps. For 0.88 M about 75% of 20 traps were closed, for 8.82 M about 85% of 20 traps were closed. Drops of distilled water instead of hydrogen peroxide placed on the midrib of the trap. After a few seconds, about 80% of traps, no direct electrostimulation by plasma is involved. RONS can activate ion channels [37,38] and ion transport in membranes of the traps, no direct electrostimulation by plasma is involved. RONS can activate ion channels [37,38] and ion transport in membranes of the traps, no direct electrostimulation by plasma is involved. RONS can activate ion channels [37,38] and ion transport in membranes of the traps, no direct electrostimulation by plasma is involved. RONS can activate ion channels [37,38] and ion transport in membranes of the traps, no direct electrostimulation by plasma is involved.

4. Discussion

Why did cold plasma cause such effects on the plants? Which plasma components are responsible for the observed plant activation?

Our simulations of gas flow using commercial CFD software showed that gas velocity of the argon jet does not exceed 5 m/s (Fig. 4b). Such a low flow speed cannot affect our plants. Indeed, we have directly tested that the argon jet in the absence of plasma does not produce plant activation.

To evaluate effects of plasma radiation, we have placed plants next to the discharge tube, where visible and UV radiation both have maximal values. No effect on the plants has been observed.

To evaluate effects of electric fields and ions, we have changed the distance between the plant surface and the jet exit. It is known that plasma jets in He or Ar gas exist in the form of an ionization wave (plasma bullet) traveling with velocities in the range of 10⁷–10⁸ m/s, which are orders of magnitude larger than the gas flow velocities [31]. The maximal densities of electrons and ions inside an argon plasma bullet measured under similar conditions have maximal values of 10¹⁷ m⁻³ and electron temperatures do not exceed 4.5 eV [32]. The densities of charged species decay rapidly outside the bullet due to volume recombination. Thus, “plasma bullets”, i.e. guided ionization waves propagating along the plume deliver net zero charge to the plants but could induce electric field splashes associated with propagation of ionization fronts. Since the effects are induced by the tails of the plume and do not change significantly with decreasing distance to the plume, we believe that electric fields are not the main cause of the observed effects. Thus, we believe that long-living active neutral species produced by cold plasma are responsible for the observed effects. The typical active species produced by a cold plasma jet include O, O₂, O₃, O₂, OH, H₂O₂, NO, NO₂, N₂O₅, and N₂O. Most of these RONS are known to interact with living matter and have been studied by the free radical biology community [33]. The actual composition of the RONS cocktail depends on the plasma gas mixture, the discharge type, and the operating conditions.

RONS are involved in signal transduction and cell death [4,6,15]. The specific biological response of a plant to RONS depends on the chemical identity of the RONS, the intensity of the signal, sites of production, the plant developmental stage, previous stresses encountered, and interactions with other signaling molecules such as nitric oxide, lipid messengers, and plant hormones. The most important ROS radicals include superoxide, hydroxyl, hydroperoxyl, hydrogen peroxy, peroxyl, and singlet oxygen. These species are cytotoxic to plants and can lead to cell death [34]. For example, the reaction of nitric oxide with the superoxide radical produces a short-lived oxidant peroxynitrite, which is a potent inducer of cell death [35].

Fig. 5 shows the proposed mechanism of closure of the Venus flytrap by mechanical stimulation of trigger hairs (E) or by cold plasma (F). It is known that the lobes of the Venus flytrap move because of the water flux across biological membranes driven by osmosis [19]. The water flow occurs as passive diffusion across the lipid bilayer [36] facilitated by aquaporins, which may play a pivotal role in osmoregulation in plants. The cold plasma treatment of lobes, midrib or cilia induces the trap closure without triggering the mechanosensitive channels. In previous experiments, the trap was closed by direct electrostimulation with positive electrodes in a midrib and negative electrodes in a lobe [21–25]. Since cold plasma can close traps by stimulation of any part of the traps, no direct electrostimulation by plasma is involved. RONS can activate ion channels [37,38] and ion transport in membranes [38], which can induce osmotic water flow through aquaporins and plant morphing [21]. Thus, RONS can be the primary reason of the trap’s closing by cold plasma.

Plasma treatment of Mimosa pudica induces plant movement similar to stimulation of mechanosensors by the mechanism shown in Fig. 6. It is well known that reactive oxygen species [38] or electrical signal transduction to pulvin [26–29] can activate cation channels in plants [38]. The activation of voltage-gated ion channels induces redistribution of ions between extensor and flexor layers of the pulvin [26–29]. The differential volume changes of the flexor and extensor sites are caused by the transport of ions accompanied by osmotic transport of water. Ion fluxes activate fast water transport through aquaporins between the lower half and the upper half of the pulvinus.

5. Conclusions

We have observed for the first time that argon plasma jet induces morphing of Venus flytrap and Mimosa pudica. We have evaluated possible contributions of different plasma components on the plant activation and concluded that plasma-generated ROS should be responsible for the observed effect. Direct experiments have confirmed that drops of H₂O₂ on the midrib (common ROS in biosystems) can induce closing of the Venus flytrap similar to that induced by cold plasma.

The present work offers new insight into bioelectrochemical mechanisms of plant activation and movement. Cold plasma treatment of lobes, midrib or cilia induces the trap closure without triggering the mechanosensitive channels.
Further studies of the underlying mechanisms could promote plasma technology for plant developmental control and future use for plant protection from pathogens, as well as from biotic and abiotic stresses on crop plants. Understanding plasma control of plant morphing could help in designing adaptive structures and bioinspired intelligent materials.

Disclosure of potential conflicts of interest

The authors declare no competing financial interests.

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Author contributions

AGV, KCX and VIK designed and performed experiments. All authors recorded and evaluated plants responses to cold plasma activation. All

Fig. 5. Mechanisms of the Venus flytrap activation. Morphing structures in the Venus flytrap induced by stimulation of mechanophytosensors (left) and by direct stimulation of the trap by cold plasma (right): open trap (A); mechanosensitive trigger hair (B); closed trap (C); locked and constricted trap (D).

Fig. 6. Morphing structures in Mimosa pudica induced by stimulation of mechanosensors (A) or by plasma stimulation of a pinnule or a pulvinus without stimulation of mechanosensors (B).
authors wrote the manuscript. All authors read and approved the manuscript.

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