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Nonthermal Plasma Reduces Water Consumption While Accelerating *Arabidopsis thaliana* Growth and Fecundity

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ABSTRACT: With climate change and increasing world population, the competition for water available for crop irrigation has increased. The main methods employed to meet the existing food needs are addition of fertilizers to soil and genetic engineering of plants. However, the short- and long-term impacts of these techniques on health and environment are of major concern. The study presented here demonstrates that nonthermal plasma (NTP) treatment of water may address these challenges without the addition of chemicals. Plasma produces a wide variety of metastable radicals, predominantly reactive oxygen and reactive nitrogen species (ROS, RNS) that have been previously proven to activate plant defense responses and to accelerate growth. In this work, NTP was used to treat deionized water for irrigation of *Arabidopsis thaliana* plants for 7 weeks. Plasma treatment decreased overall water consumption for irrigation, simultaneously enhancing plant growth and yield. We suggest that the reactive nitrogen species (NO_3-N) generated by the plasma is responsible for the increased fecundity of plants.

KEY WORDS: water conservation, reactive oxygen species, reactive nitrogen species

I. INTRODUCTION

It is estimated that by 2025 at least 1.8 billion people will be affected globally by absolute water scarcity. To address this challenge, the United Nations declared 2005–2015 as the International Decade for Action "Water for Life." It is anticipated that domestic, industrial, and livestock water use will increase by 50%, limiting the availability of water for irrigation. Growing population, increasing effects of climate change, and uneven distribution of water are some of the root causes for global water scarcity. Other contributing causes of physical deprivation of water include socioeconomic relations, political powers, poverty, and inequality.[1] It is estimated that almost 25% of the world population is deprived of water resources required for sustaining agricultural efforts.[1] There is an urgent need to develop innovative techniques that are affordable and environmentally friendly to sustain agriculture without compromising the plant's health and yield. Our research focuses on nonthermal plasma (NTP), which is one such emerging field in eco-agricultural technology.

Plasma, often referred to as the fourth state of matter, is an ionized gas containing

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charged species, excited atoms and molecules, and various forms of radiation. Plasma is widely used as an active catalyst for many important chemical processes, such as deep ditch etching in microelectronics, as a light source in plasma TVs, and recently, various biomedical applications where plasma is applied directly to biological tissues.[2] In agriculture, plasma has been shown to stimulate plant growth by enhancing seed germination in soybeans.[3] Plasma treatment has also been successful in enhancing germination and breaking dormancy of wheat and oats, usually caused by drought and high temperatures.[4]

The antimicrobial effect of plasma has also been explored for its uses in agriculture. In 2014, Panngom et al.[5] demonstrated that direct NTP treatment of *Fusarium oxysporum* fungal spores compromised their ability to infect tomato plants. On the other hand, treatment of the roots of tomato plants upregulated expression of pathogen resistance genes.[5] In another study, Selcuk et al.[6] used NTP to inactivate and eliminate the pathogenic fungi *Aspergillus* spp. and *Penicillium* spp., responsible for spoilage of stored grains and legumes, from contaminated grains. Plasma treatment of tomato seeds was also demonstrated to increase their resistance to *Ralstonia solanacearum* and improve both germination and plant growth.[7]

Recently, plasma systems have been used to treat water, enriching it with chemical compounds produced by the plasma. This plasma-activated water has been previously shown to effectively decontaminate and disinfect plants, and produce other beneficial effects.[8] In 2013, Park et al.[9] demonstrated that plant growth and yield improved when spinach, tomato, and pepper plants were irrigated with NTP-treated water. The authors suggest that this effect may be a result of the lower pH and increased levels of nitrates measured in this water. NTP also generates transient metastable peroxides, acids, and salts in water,[8,10] providing conditions that enhanced plant growth.

We hypothesized that plasma treatment of water would decrease the amount of water needed for irrigation without compromising the plant development and fecundity. Hence, the goal of this study was to determine whether the use of NTP-treated water for irrigation of plants could provide multiple benefits without extra energy costs. We demonstrated that water consumption per plant was reduced when irrigated with NTP-treated water, especially in the early developmental stages. The low consumption of plasma water did not compromise plant growth and yield. This could be a simple and cost-effective strategy for conservation of irrigation water that woul support sustainable agriculture with only minor alterations in infrastructure and existing practices. NTP treatment may also allow possible use of recycled water for irrigation.

II. MATERIALS AND METHODS

A. Plasma Treatment of Water

Nonequilibrium gliding arc plasma in air was used to treat the water on the same day it was administered to plants under observation. This NTP reactor is a cylindrical vessel with a diameter of 1 inch, and it has a tangentially positioned air injection site between

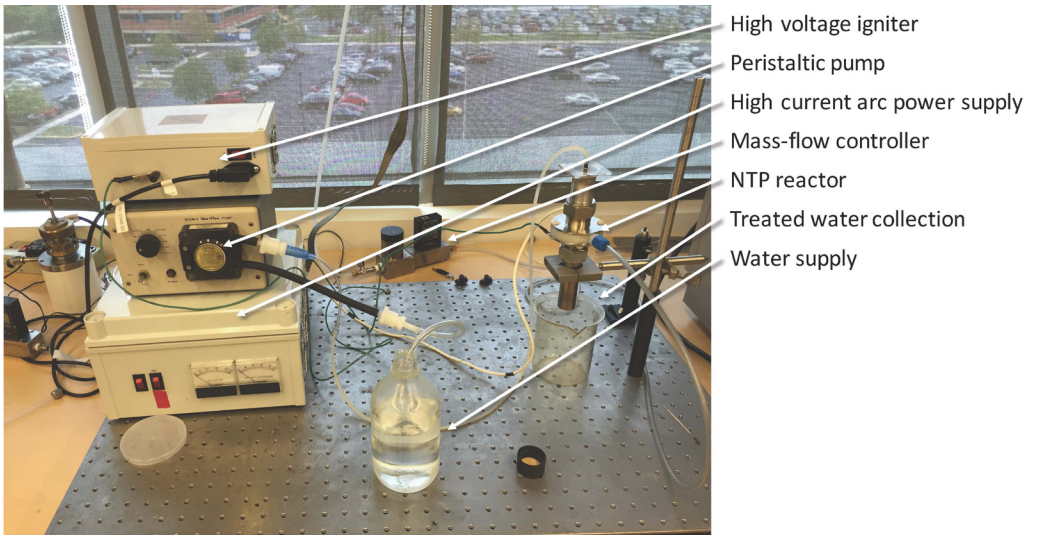


FIG. 1: Experimental setup for plasma treatment of water

two electrodes housed within the vessel (Fig. 1). The tangential air injection site located between the high voltage electrode and the grounded electrode allowed the plasma discharge to occur at the air inlet and propagate in a vortex geometry along the walls of the cylindrical reactor. The effect of geometry on thermal insulation and discharge breakdown of a similar three-dimensional vortex gliding arc configuration have previously been described by Kalra et al.[11] Current and voltage capabilities of the power supply used to form the discharge are generally load dependent, but the average power consumption for the plasma regimen used in this study was 47 W. This results in energy consumption of 0.01 kWh per 1 L of water treated. Compressed air was delivered to the reactor through the described injection site using a mass flow controller at a rate of 28.3 SLPM. A peristaltic pump was used to maintain the flow of autoclaved water through the plasma reactor at a rate of 80 mL/min. Water was injected through capillary tubing concentrically placed at the inlet of the cylindrical vessel. In this setup, once the water exits the tubing it mixes with the plasma and is then collected in autoclaved beakers at the exit of the reactor.

This NTP treatment generated water containing more than 100 ppm of O_2^-, 40–80 ppm NO_2^-, and 100–250 ppm NO_3^-. The water generated from this treatment had a pH of approximately 3–4. The NTP treated water was delivered to the plant incubation site within 1 hour after treatment.

B. Germination and Growth Conditions for Plants

Wild-type *Arabidopsis thaliana* (Columbia-0) seeds were sterilized in a mixture of 30% bleach and 70% alcohol before plating for germination on Murashige and Skoog (MS)

medium to ensure uniform germination. After 12 days, the seedlings were transferred into pots containing Sunshine Mix (Sun Gro Horticulture, Quincy, MI) and incubated in an environmental growth chamber maintained at 24°C with 70% relative humidity and a 12-hour photoperiod. The next day, plants were randomly separated into two groups of 24 plants each: irrigated with autoclaved deionized water (control) or with autoclaved deionized water treated with plasma. All plants were irrigated with a measured volume of water, twice a week to achieve 90% soil moisture level as measured by a moisture meter (Mudder 3-in-1 Soil Moisture Meter). Water was added to the outer tray that contained the individual pot inserts. Soil moisture levels were measured immediately before watering to evaluate water loss.

C. Water and Soil Analysis

The pH of the plasma treated water and control water was measured with indicator test strips (Millipore, 109535) before and after irrigating the plant pots. To measure nitrate (NO_3^-) and nitrite (NO_2^-) content, 1 g of soil was mixed with approximately 10 mL of spring water, free of NO_3^- and NO_2^-. Test strips were used to assess nitrate levels before and after irrigation. Three independent pots were measured for statistical significance.

D. Statistical Tests

Observations were taken for 7 weeks. Five independent observations were taken from every treatment and these observations were randomized. Student's *t* test and one-way analysis of variance (ANOVA) was used to test significance.

III. RESULTS

A. Consumption of NTP-Treated Water by *A. thaliana*

Total consumption of water was significantly reduced in plants irrigated with plasma-treated water (Fig. 2). Over the 5-week irrigation period, plants irrigated with NTP-water were fed 8.3 L of water, while control plants received 9.5 L. Most of the effect was seen in weeks 3–4, when a 20%–30% reduction in water consumption was observed in plants that were hydrated with NTP-water. There was no major difference between the groups during weeks 4 to 5. Despite lower water consumption, the plants grew larger.

B. Effects of NTP-Water on Leaf Size of *A. thaliana*

Fig. 3a shows the leaf area of plants as observed from week 3 to week 6. Plants in the control group had significantly smaller leaf sizes ($p < 0.05$), as compared to the plants treated with plasma water. Growth was evaluated by following changes in leaf size over the period of observation. Image J (http://imagej.nih.gov/ij/) was used to measure leaf size from images of individual plants, taken at weekly intervals. Results are presented

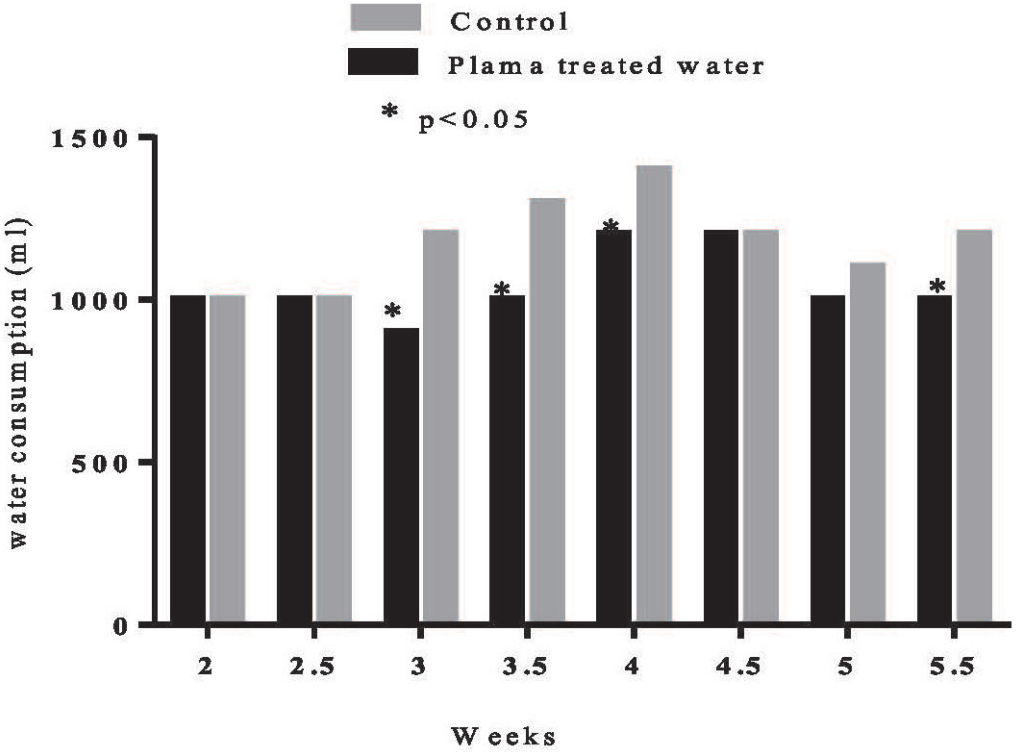


FIG. 2: Water consumption of plants watered with deionized (DI) or plasma-treated DI water

in Fig. 3. Each bar represents the average measurements from three randomly chosen leaves from three different plants. Absolute growth rate (AGR) of plants, which reflects growth per unit time,[12] was determined for plants watered with control or NTP water. The relative growth rate (RGR), on the other hand, peaked at week 4 for all groups. This suggests that the life cycle of the plants was unaffected. During the vegetative development stage, there was no difference in the number of leaves in the rosette; and there was no difference in the radial symmetry of the rosette for either the control or the treatment groups.

C. Effects of NTP Treated Water on Flower and Seed Production

In the reproductive stages, flower and seed production were used as indicators of the effect of NTP water on fecundity of *A. thaliana*. Flower and seed numbers were counted for five plants at week 7 of development. Control plants had significantly fewer flowers and seeds than plants irrigated with NTP water. The 24 plants treated with NTP water produced a total of 347 flowers between weeks 5 and 7. In contrast, the control group produced a total of 181 flowers during the same period (Figs. 4 and 5).

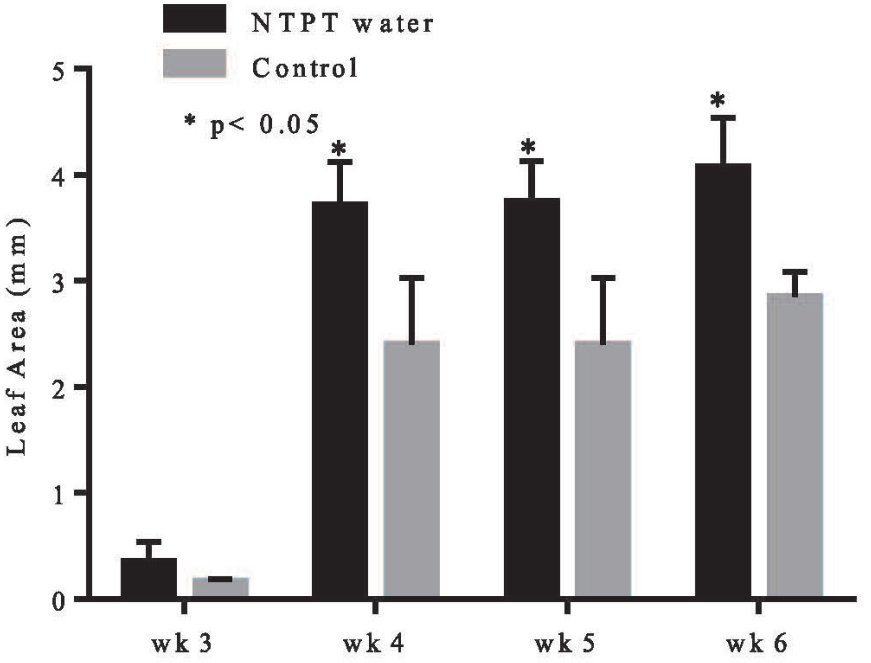


FIG. 3a: Leaf area calculated with ImageJ for leaves from control and plants watered with NTP water. The values represent an average of three leaves ±SD

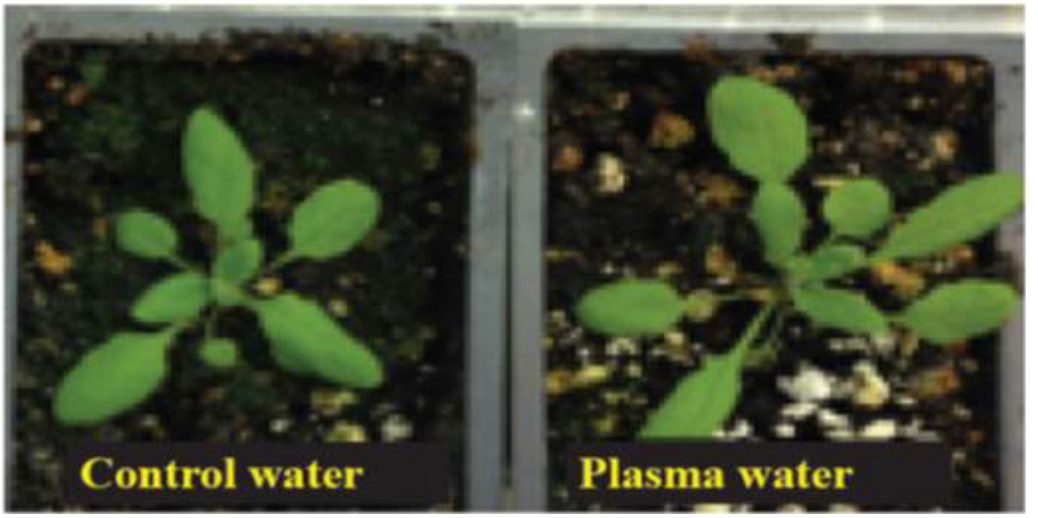


FIG. 3b: Difference in size of the plants watered with plasma water compared to controls. Plants in the picture represent 4-week-old plants

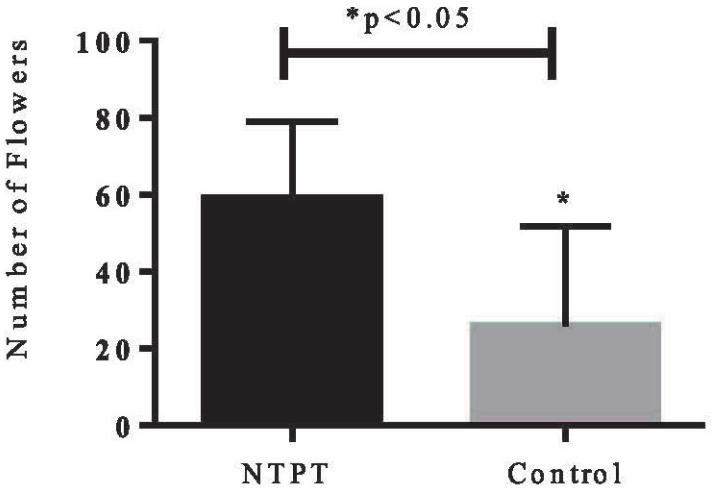


FIG. 4: Week 7 flower yield (n = 5).

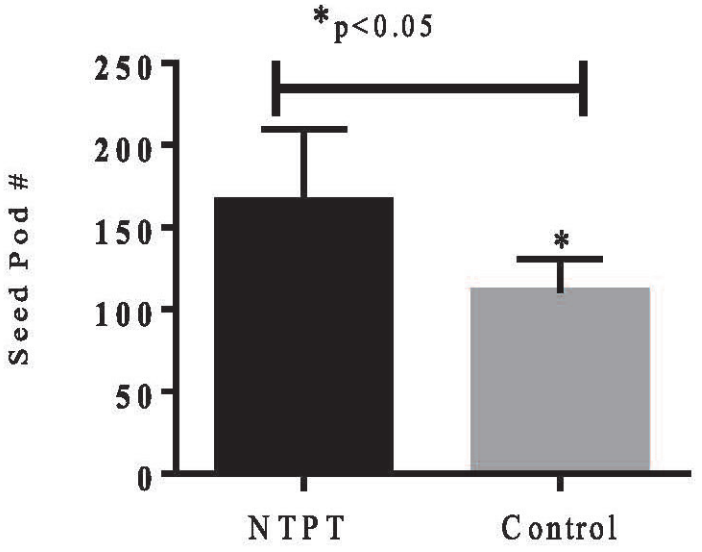


FIG. 5: Week 7 seed yield (n = 5).

D. Nitrate and Moisture Content in Treated Soil

To determine residual nitrate levels in the soil, nitrate strips were used before the addition of irrigation water, representing NO_3^-–N species because there was no NO_2^- detected in the NTP water or in the soil. Soil treated with NTP water had 2.5-fold higher levels of nitrates from week 3 to week 5 compared to the control plants (Fig. 6a), a

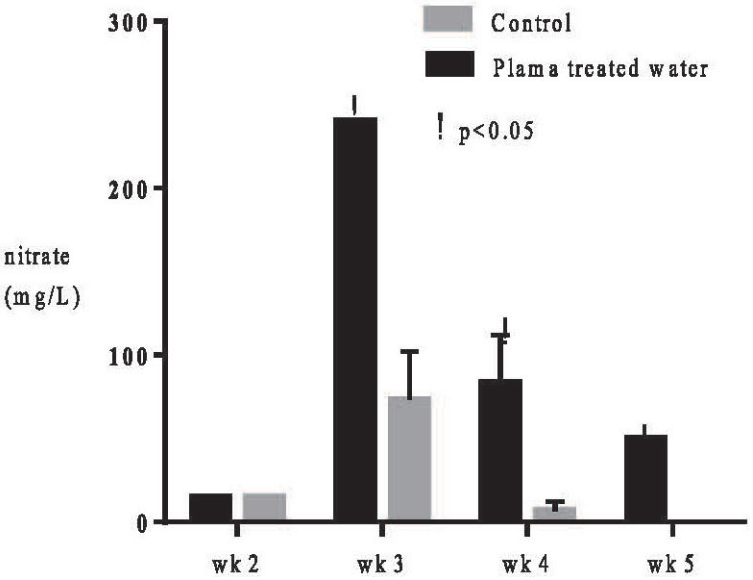


FIG. 6a: Nitrate levels in soil measured prior to irrigation of plants from weeks 2–5 of development (n = 3).

possible contributor to enhanced plant growth. Subsequently, these nitrates significantly decreased in the soil three days after the addition of water, suggesting nitrate uptake by plants (Fig. 6b). Nitrate is the most important form of nitrogen in soil. Nitrate uptake by roots and its transport through the whole plant impacts the overall performance of the plant.

In a comparative study of the moisture content of soil, we found that soil irrigated with NTP water retained water better for the first three weeks of development. In the later weeks of development, there was no significant difference in the moisture content. Nevertheless, water consumption by plants irrigated with NTP water remained lower than that of the control samples.

IV. DISCUSSION

Irrigated agriculture accounts for about 70% of global water consumption and is the second largest user of water in the USA. Our study demonstrates that NTP treatment reduces the water consumption by approximately 30% and results in higher plant yield. Recent developments in plasma technology have made the production of plasma-activated water a cost-effective procedure.[10] Additionally, the transient nature of the plasma-generated metastable species results in no long-term environmental impact because these species revert to O_2 and H_2O (Fridman and Fridman, 2012[2]).

This study demonstrates that plasma treatment markedly decreases the consumption of water with an associated increase in growth indices and fecundity (i.e., leaf size

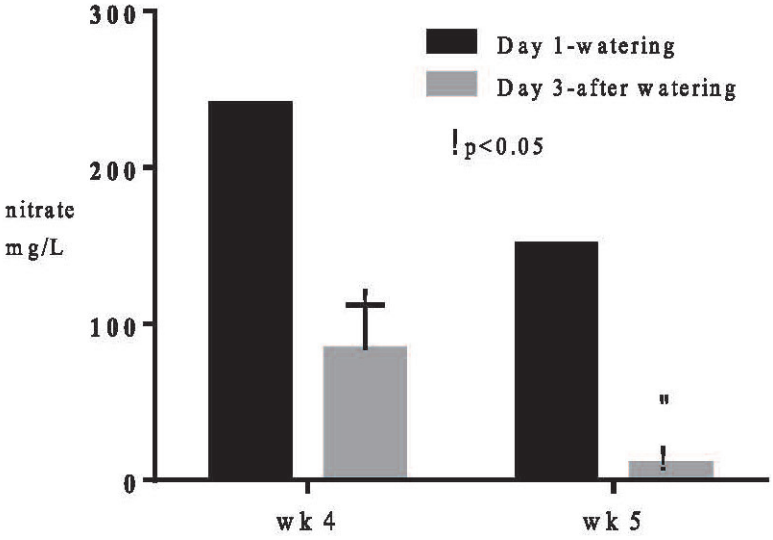


FIG. 6b: Measurement of nitrate in the soil of plasma treated watered plants immediately after watering and 3 days later (n = 3)

and flower and seed production). Recent reports have shown similar growth effects on oilseed and rapeseed seedlings that were treated directly with cold plasma.[13] However, the advantage of our approach is that the water used for irrigation of plants is enriched by plasma, resulting in reduced water consumption as well as better plant performance.

Treatment of water with atmospheric pressure plasmas results in generation of reactive oxygen and reactive nitrogen species (ROS, RNS). RNS include radicals such a nitric oxide (NO), nitrite (NO_2^-), nitrate (NO_3^-), and nonradicals such as nitrous acid (HNO_2).[8] Nitric oxide is known to be a key signaling molecule in plant growth, development, senescence, and in plant response to environmental stress.[14] Therefore, the observed increased growth of plants irrigated with plasma-treated water could be attributed to these species; however, this still remains an open question in need of further investigation.

Nitrogen radicals also lower the pH of NTP-treated water to a range of 5–6. The desirable pH range in the root zone for most plants is 5.5–6.5. To achieve this pH level, many growers typically add acid to their irrigation water, but plasma water is already acidic. Nitrate levels of soil increased immediately after the addition of NTP water but revert to baseline levels by day 3. These nitrates may have been sequestered by the plants in this study, which resulted in an increase in biomass as evidenced by the larger leaf area and the increased yield of flowers and seeds. Additional experiments are required to monitor the assimilation of nitrates into the plant.

ROS generated in plasma-treated water include oxygen radicals, such as superoxide (O_2^-), hydroxyl radical (OH), and nonradicals such as hydrogen peroxide (H_2O_2).[8] These serve as signaling molecules in cells and finely modulate the plants to activate defense

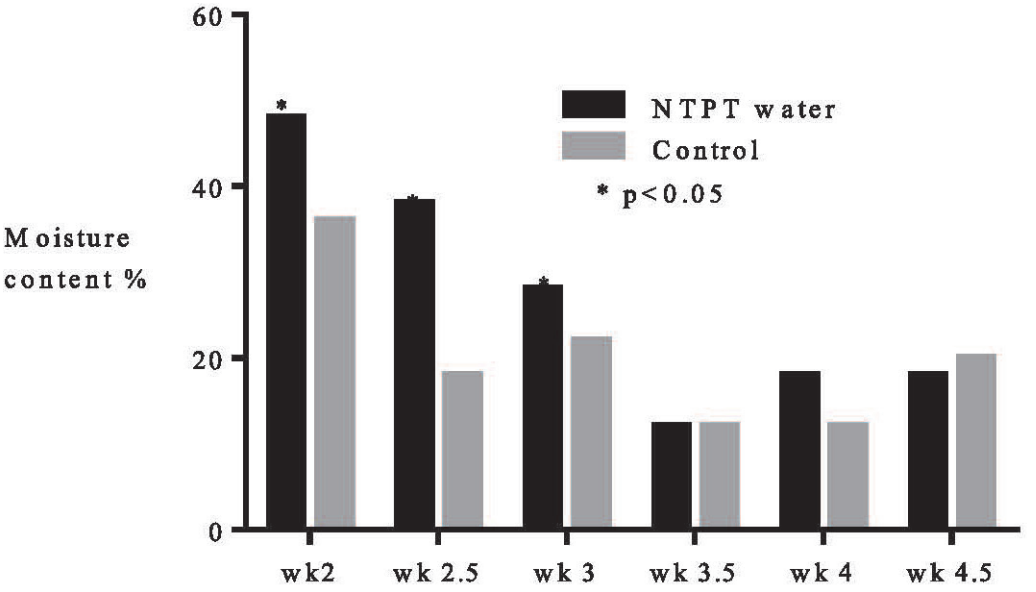


FIG. 7: Moisture content of soil irrigated with NTP water and control (n = 5)

responses, resulting in increased plant growth responses.[15] Generally, ROS are produced in different cell compartments in plants, but these are scavenged by antioxidant enzymes present in plants, and this supports cell homeostasis.[14] However, a recent study by Ling et al.[13] in 2015 showed that plasma treatment stimulated superoxide dismutase (SOD) and peroxidase (POD) activity in rapeseed seedlings. Similarly, increased activity of SOD and POD also occurred in tomato seedlings treated with plasma.[16] The increase in defense responses of plants due to ROS in plasma-treated water could also contribute to the effects we observed on growth and fecundity. ROS are known inducers of factors related to stress response, such as mitogen activated protein kinases (MAPK), transcription factors, antioxidant enzymes, dehydrins, heat shock proteins, and pathogenesis-related proteins.[17] Hence, the modifications in water chemistry from plasma treatment may produce the observed effects on plants either (1) *directly* by affecting the existing cellular factors, or 2) *indirectly* by affecting gene expression of important regulatory and stress factors.

The retention of moisture in soil irrigated with plasma-water (Fig. 7) may be a result of reduced soil hydrophobicity caused by alterations in the chemical structure of the surface of the substrate.[18,19] This could facilitate penetration of water into the smaller pores of the substrate and enhance distribution of water and nutrients, thus reducing the need for frequent irrigation. However, further studies to evaluate changes in the hydrophobicity and soil chemistry are required to validate this assumption. Hence, there is a possibility that the lower consumption of water could be due to changes in soil chemistry that in turn affects plant development and yield.

V. CONCLUSIONS

Plasma treatment reduced water requirement of *A. thaliana* during the observation period reported here. This was associated with increased plant biomass and yield. The results of our study present an innovative approach for conservation of water used for irrigation of plants. Engineering challenges for application in large-scale farms will require scale-up of systems, validation of robustness and efficacy, and evaluation of safety. Further biological studies are required to ensure effects on plant physiology, nutritional content, and long-term effects on plant biology.

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