Plasma activated water generation, characterization and application for seed germination and plant growth

Harsha Rao¹, Punit Narayanappa¹, <u>Lakshminarayana Rao¹</u>, Anand M. Shivapuji¹ and Dasappa S¹

¹Plasma Lab, Centre for Sustainable Technology, Indian Institute of Science, Bangalore, Karnataka, India

Abstract: In recent days, there is tremendous scope for plasma activated water (PAW) based agriculture specifically, its application for enhanced seed germination and plant growth. Here we report on a very low cost, scalable design of PAW system. The PAW generated is characterized for its reactive nitrogen species and its application for enhanced seed germination and plant growth is characterized. The PAW shows a significant effect on seed germination and plant growth due to reactive nitrogen species present in the plasma activated water

Keywords: Plasma activated water (PAW), Total nitrogen species, Root length, and Shoot length

1. Introduction

Plasma activated water (PAW) can be produced by exposing a column of water to cold plasma [1, 2]. It is well known that PAW contains reactive oxygen and nitrogen species, because of which it has application in enhanced seed germination and plant growth. According to Food and Agricultural Organization of United Nations, over the next decade, the world food shortage is likely to increase by three times likely due to industrialization, urbanization and climate change, as the yield starts reducing and the land remains almost the same. By using fertilizers the plant growth and yield can be increased but only at the cost of increased pollution due to use of excessive fertilizers [1, 2]. Nowadays, use of PAW as alternative for fertilizers is showing a lot of promise. In this paper we report on a very low cost, scalable design for generating PAW using commercially available components and tap water. The PAW generated is characterized for its reactive nitrogen species and its application for enhanced seed germination and plant growth is reported. The effect of PAW on seed germination was measured using green gram, maize and plant growth using tomato seeds.

2. Materials and Methods

2.1 Plasma activated water generation DBD method

Figure 1 shows the plasma activated water generation setup used in this study. The setup consisted of open ended, insulted, coiled metallic wires which acted like plasma forming electrodes. A commercially available lighting 25 W power supply having an OCV of 4 kV and discharge frequency of 25 kHz was used as plasma power supply. A lab glass bottle was used to generate PAW. The coiled wires were embedded into the cap of the glass bottle as shown in figure 1.

To generate PAW, a known quantity of water (about 100 ml) is taken in the glass bottle and the cap with the electrodes was placed on the bottle. The power supply

was switched on for a fixed time length (say 20 mins). At the end of the 20th minute, the power supply was switched off and the cap with electrodes was replaced by a regular cap. The contents of the bottle were well mixed manually to obtain PAW. Tap water was used for to generate PAW. The nitrate and nitrite content of the PAW was measured.

The total nitrogen in PAW (Nitrate+Nitrite) was measured using standard cadmium reduction method using spectrophotometer measurement technique at wavelength of 500 nm and is reported as NO₃-N. Nitrite is measured using standard USEPA Diazotization Method using spectrophotometer at wavelength of 507 nm.

2.2 Seed germination studies

Filter paper method was used to measure seed germination using PAW and its performance was compared against a control, which was tap water (TW). Seed germination experiments were conducted using green gram and maize as seeds. 200 seeds each were used i.e., germination behaviour of 200 seeds with PAW was measured and was compared with the germination behaviour of 200 seeds with TW. For germination studies, 10 seeds were placed in a 90mm petri-dish plate with filter paper as supporting layer. The seeds were kept moist by replacing about 4 ml of water in these petri-dishes. Germination was studied for 5 days. The root length, germination germination potential, percentage, germination index, length index were measured daily for 5 days and average root length of seeds was measured using a ruler with a precision of 1mm.

The formula used for germination parameters [1] are

Germination % (G%) = $\frac{\text{total number of germinated seeds}}{\text{total number of seeds}}$

 $Germination potential (GP) = \frac{number of seeds germinated in 3 days}{total number of seeds}$

Germination Index (GI) = $\sum \frac{number \ of \ germination \ seeds \ in \ t \ days}{germination \ days}$

Length Index (LI) = GI * total length (L)

2.3 Plant growth

Cup method was used to study the effect of PAW for plant growth using tomato seeds and its performance was compared with the control which was TW. 20 seeds were used with 4 seeds each in 100 ml paper cup with 30 gram of coco peat as the support material in the cup to grow tomato plants. The plants were exposed to semi sun light with a feed of 10 ml of water once every 2 days, for a period of first 2 weeks and 20 ml of feed water once every 2 days for the rest of 4 weeks. The growth parameters like length of shoot, root, number of leaves, and number of nodes (branches) were measured after 46 days using a ruler with precision of 1mm. The number of leaves were counted at the end of 46 days.



Figure 1: (a) Plasma streamers on electrode (b) Plasma activated water generator setup

3. Results and Discussion

- **3.1** Plasma water characterization
- > TOTAL NITROGEN SPECIES in PAW:

The total nitrogen species (TNS) namely nitrate, nitrite in nitrogen form is measured in PAW. The TNS concentration increased almost linearly with activation time as shown in figure 2. The concentration of the TNS increased from 2.5 to 22 mg/l for 100 ml of water as the activation time increased from 0 to 20 min

> NITRITE CONCENTRATION in PAW

Figure 3 shows the increase of nitrate in PAW with activation time. The concentration varies almost linearly with activation time as shown in figure 3. The Nitrite in nitrogen is increased from 0 to 0.025 mg/l as the activation time increased from 0 to 20 minutes.



Figure 2: The total nitrogen species (NO3-N + NO2-N) variation with activation time for Tap and DM water.

These results are attributed to the generation of nitrogen species in the head space above the water column in the bottle and its subsequent transfer to the water during mixing of the bottle. The plasma discharge, in the head space above the water column generates nitrates and nitrites which gets dissolved in to the water thereby increasing the nitrate and nitrite level in the PAW.

3.2 SEED GERMINATION in PAW

A 20 min of tap water activated PAW shows more increase in the average root length and length index in comparatively with tap water is shown in table 1 & 2 and figure 4 and 5. The length index (mm) is a measure of root length over the period of 5 days and the average length is measured daily for each seed for a period of 120 hr with an almost fixed interval of time.



Figure 3: The Nitrite in Nitrogen concentration variation with activation time for Tap and DM water

Table 1 shows the seed germination results of PAW and TW for both green gram and maize seeds. As can be seen from table 1, though the germination index and

percentage were almost the same for green gram and maize, the length index was higher in PAW when compared to TW. That is, seeds fed with PAW germinated to a longer length and looked healthier when compared to that of TW. Also, we noticed that PAW fed seeds germinated faster when compared to TW. This result is is mainly attributed due to the nitrate present in PAW as observed by main researchers [1-3]. The plasma induced nitrate present in PAW will result in enhanced germination of seeds relative to TW which does not have the additional nitrates. It is well known that the presence of nitrate in the water will result in hormonal variation i.e., the ABA and GA hormones present in the seeds will be effected by nitrate which will lead to reduction in dormancy of the seed [5] resulting in enhanced seed germination.

Table 1: Seed germination parameters for Green gram

Green gram						
	G%	GP	LI	GI		
TW	98	98	7537	425		
PAW	99	99	13013	438		
Maize						
TW	95	95	7026	237		
PAW	97	97	12062	248		

Figure 4 and 5 shows the average root length measured for green gram and maize. As can be seen from these figures, the PAW fed seeds had the root length were about 1.5 times more in comparison with TW for both green gram and maize. The difference in the average root length was significant from 3 days itself. The pictures germinated seeds is shown in figure 8 and 9 for green gram and maize respectively after 5 days.



Figure 4: Average root length (mm) variation of green gram over a period of 120 hr



Figure 5: Average root length (mm) variation Maize over a period of 120 hr



Tap waterPlasma activated waterFigure 6: Picture of germinated green gram after 120 hr



Tap waterPlasma activated waterFigure 7: Picture of germinated Maize after 120 hr

3.3 PLANT GROWTH in PAW

The PAW shows enormous growth in the average root, shoot, nodes, and a number of leaves in comparisons with tap water as shown in Table 2 and figure 8. The average shot length, root, leaves was almost doubles with respect to tap water as shown in table 2 and figure 8. The increase in the leaves and nodes of PAW fed plants is attributed to the presence of nitrate in paw. Nitrates are known as an important constituent of chlorophyll. The Nitrogen is an important building block for protein and amino acids synthesis and helps in the germination of the plant.

	shoot length [mm]	root length [mm]	Nodes [#]	Leaves [#]
TW	3.68±0.6	9.99±5	2.15±0.7	2.5±0.6
PAW	5.385±0.9	17.39±6	3.1±0.7	8.8±1

Table 2: Plant growth parameters for Tomato



Figure 8: Pictorial view of tomato plant growth in 46 days

Conclusions

In this paper, we were able to develop a very low cost PAW system to activate tap water using commercially available off the shelf components. We believe that the design is easily scalable and can be implemented successfully in agricultural systems especially one using drip irrigation methods. We have shown that with limited exposure of plasma to the water, the reactive nitrogen species in the water is increased. This increase was linear with activation time and can be used to precisely control the amount of nitrogen that can be supplied to the plants at various times of their growth. We have also demonstrated that the PAW results in enhanced seed gemerinaton and increased plant growth and can be used as an alternative to conventional fertilization practices.

4. References

- [1] R. Zhou, R. Zhou, *Sci. Rep.*, vol. 6, no. September, pp. 1–11, 2016.
- [2] L. Sivachandiran, RSC Adv., vol. 7, no. 4, pp. 1822– 1832, 2017.
- [3] A. Zahoranová, *Plasma Chem. Plasma Process.*, vol. 36, no. 2, pp. 397–414, 2018.

- [4] C. Lo Porto, *Innov. Food Sci. Emerg. Technol.*, vol. 49, no. July, pp. 13–19, 2018.
- [5] R. Zhou, Innov. Food Sci. Emerg. Technol., no. August, pp. 1–9, 2018.