



Enhancement of Maize (*Zea mays* Var: Gs-2) Plant Growth and Yield: Seed Treatment with Non-Thermal Plasma using Different Gases

KUNDAN VILIYA^{1*}, UTTAM SHARMA¹, MANISHA THAKUR², KADUR NARAYAN GURUPRASAD², JAYSHREE SHARMA³, RAMKRISHNA RANE⁴, AMULYA SANYASI⁵ and JOYDEEP GHOSH⁵

¹Department of Physics, Shri Vaishnav Institute of Science, SVVV Indore, India.

²Department of Life Science, Shri Vaishnav Institute of Science, SVVV Indore, India.

³Department of Physics, Shri Vaishnav Institute of Management, DAVV, Indore, India.

⁴Facilitation Centre for Industrial Plasma Technologies, Institute for Plasma Research, Gandhinagar, India.

⁵Aditya-Upgrade Tokamak Operations Division, Institute for Plasma Research, Gandhinagar, India.

Abstract

Impact of pre-sowing treatment of maize (*Zea mays* Var: GS-2) seeds with non-thermal glow discharge plasma on the growth and yield of field grown plants has been investigated. Maize seeds were treated with non-thermal glow discharge plasma produced from five different gases Nitrogen (N₂), Helium (He), Argon (Ar), Hydrogen (H₂) and Oxygen (O₂) before sowing the seeds in the field. Seeds were exposed to 30 W for 240 seconds to glow discharge plasma produced in a cylindrical stainless steel vacuum chamber utilizing an automatic matching network and a 13.56 MHz with the capacity up to 600W RF power source are used. Plasma treated seeds were grown in the field and growth measurements were made at 30, 60 and 90 days after sowing. Pre-sowing treatment with glow discharge plasma enhanced plant height, leaf length and plant fresh weight at all the stages of growth. O₂ and N₂ plasma treated seeds were more effective than He, H₂ and Ar in enhancing the growth and biomass of the plants. The yield parameters are cob length 21% in O₂ and 19% in N₂, cob weight 36% in O₂ and 34% in N₂, and 100 seeds weight 39% in O₂ and 36% in N₂ plasma were significantly enhanced. It is concluded that O₂ and N₂ plasma Pre-sowing treatment can significantly enhance the biomass and yield of maize plants with O₂ slightly higher. Analyzing the characterization with scanning electron microscopy



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CONTACT Kundan Viliya ✉ viliyakundan@gmail.com 📍 Department of Physics, Shri Vaishnav Institute of Science, SVVV Indore, India.



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(SEM), and optical emission spectroscopy (OES) of maize seeds. SEM revealed considerable alterations for O₂, N₂, Ar, and He but no change for H₂ plasma treatment. The findings demonstrate that the surface of the starch caryopsis has been altered, exposing an oxygen and nitrogen plasma that has resulted in huge channels and water holes. The OES identified the presence of OH, NO, O, and N₂ radicals generated during oxygen and nitrogen plasma treatment, which is contributing to the best result.

Introduction

Pre-sowing treatment of seeds improves growth and yield of crop plants. Several physical and chemical methods of pre-sowing treatment of seeds have been in practice since long in agriculture. The traditional methods of treatment of seeds or priming of seeds include the physical method of scarification and chemical methods of treatment with growth regulators.¹ Treatment of seeds with magnetic fields,² and non-thermal plasma¹ have been successfully employed for improving seed germination and growth of plants. As a fourth state of matter plasma is a mixture of electrons, positively charged ions, radicals and photons from a range of energies.³ ROS (Reactive oxygen species) and RNS (Reactive nitrogen species) are the major reactive radicals produced in atmospheric air plasma.^{4,5} The plasma radiation usually consists of free radicals of oxygen like superoxide (O₂⁻) and hydroxyl (OH) radicals, ozone, nitric oxide (NO) and photons (UV). All these components have bactericidal effect and decontamination of seed surfaces is one of the reasons for the improvement of seed germination.⁶ In addition to this, chemical changes in the outer parts of the seed upon exposure to plasma are known to increase water uptake through a partially etched seed coat.⁶ Seeds absorb more water by imbibition when the hydrophilicity is increased by etching of the seed coat. Reactive oxygen and nitrogen species activate biochemical processes inside the seed which promote germination. The reactive oxygen species resulting in the formation of hydrogen peroxide and the reactive nitrogen species like nitric oxide can act as signaling molecules for the activation of amylase in starchy seeds.^{7,8} Depending on the type of the gas used to produce plasma, the promotion of seed germination and seedling growth also varies. Observed that oxygen plasma was more effective than helium plasma in promoting germination and growth of mung beans.⁹ In wheat seeds argon was more efficient in promoting

germination compared to nitrogen and air.¹⁰ There have been very few studies on the effect of plasma treatment on productivity although data is available at the laboratory level. Enhancement of productivity was seen in tomato, soybean,^{11,12,9} and corn¹³ in the final yield after plasma treatment.

An essential part of farming production is how well seeds perform, which includes how quickly they sprout, how quickly they grow, and how well they fight pathogens. Though routinely utilized, traditional seed treatments, including chemical coatings, fungicides, and manual scarification, have environmental and health hazards. Plasma treatment is a non-invasive physical technique that can be safely used for organic farming. In principle, treatment of seeds with non-thermal glow discharge plasma with different gases permitted, and currently, it is in the experimental stage. Plasma treatment does not produce any chemical or radical that is not naturally produced in the plant tissues. Plasma treatment at best changes the expression of genes involved in germination and other biological processes¹⁴ using the free radicals produced.

While research has demonstrated that plasma treatment can increase seed germination and surface sterilization under laboratory conditions, there is a lack of complete information on gas-specific effects and long-term implications. Our research examines its impact under actual field conditions.

In the present paper a detailed analysis of effect of Pre-sowing treatment of maize seeds with hydrogen, helium, argon, nitrogen and oxygen plasma has been investigated on the growth and yield of plants grown in fields under natural conditions. The chosen gases are hydrogen, helium, argon, nitrogen, and oxygen particularly relevant to seeds for several reasons. Each gas offers unique properties that influence seed physiology, storage conditions, growth, and

yield responses. This research thus seeks to expand the foundational knowledge on seed with the impact of different types of gases under various conditions.

Material and Methods

Seeds of maize (*Zea mays* var, GS-2) were purchased from Ms. Jain Seed Agency, Indore, Madhya Pradesh, India. The seeds were visually examined to confirm that they were the same size and had no evident damage or flaws. Healthy and uniform seeds were selected for Pre-sowing treatment with plasma.

Location of the Study Area

This investigation took place within the agricultural domains of the Shri Vaishnav Institute of Agriculture Science, SVVV, Indore, over the course of two successive cropping seasons in 2021 and 2022. The research site is situated at a longitude of 75.84240933836061 and latitude of 22.822125212393075. Data are taken during the month of July to October of cropping seasons in India.

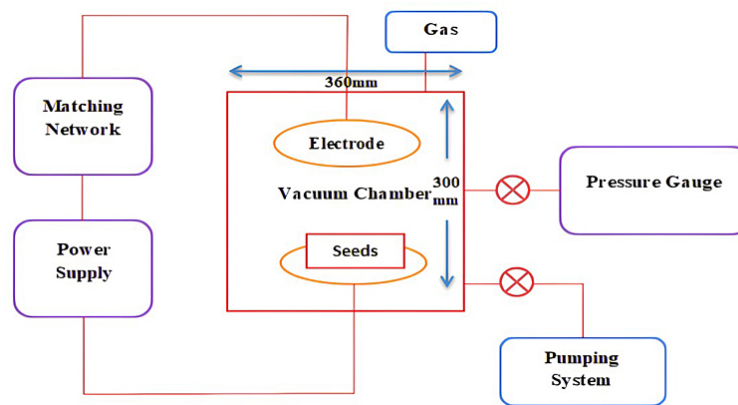


Fig. 1: Schematic block diagram of experimental setup

Plasma Treatment

The maize seeds were pre-treated using a glow-discharge plasma production apparatus using Radio Frequency power supply. In figure 1 the system comprises a cylindrical cathode assembly with an electrode-equipped stainless steel vacuum chamber. For RF electric gas discharge, an automatic matching network and a 13.56 MHz with the capacity up to 600W RF power source are used. The system consists of a rotary pump that creates a rough vacuum of 10^{-2} mbar. Various types of gases are supplied to the chamber by using a mass flow controller. Seeds were placed in the vacuum chamber between the electrodes on a pedestal with the help of a specially designed seed holder. Fifty seeds were placed on the seed holder for each exposure to plasma. After placing the seeds inside the chamber, a vacuum of 3×10^{-2} mbar was created with the help of the rotary pump. Before delivering RF power to the electrodes, one of the gases (helium, hydrogen, argon, nitrogen, or oxygen) was fed into the chamber using a mass flow controller at a flow

rate of 95 sccm. Seeds were treated with plasma for 4 minutes at 30 W. The energy level and the time of exposure were standardized, and dose response curves were obtained by varying energy levels and the time of exposure. The treated seeds were taken out of the chamber and stored in sealed plastic bags for use in the field.

Sowing and Growth and Yield in the Field

Field experiments were conducted at the agriculture fields of Shri Vaishnav Institute of Agriculture Science. Initially, we thoroughly plowed the field and made thorough preparations for planting maize. Our crop is dependent on the rainy season. A randomized block design was used for sowing the untreated or plasma treated seeds. Each row of seeds measured 30 meters long with 50 cm row to row and 30 cm seed to seed spacing. In a total of 18 rows, 3 rows were utilized for the untreated control seeds and the rest of the 15 rows were utilized for sowing seeds treated with plasma produced from five different gases (He, H₂, Ar, N₂, O₂) using 3 rows

for each gas. Throughout both years 2021 and 2022 of the crop season, the overall precipitation ranged between 40 and 45 inches. Growth measurements were made at 30, 60 and 90 days after sowing. Growth data presented is an average of 5 plants and yield data is average of ten plants. The field data presents the average percentages from both 2021 and 2022. However, for a more detailed breakdown, the comprehensive data is provided in Tables 1, 2, 3, and Figures 2, 3, and 4. For all the graphs, the X-axis labels represent different types of plasma: 1 and 6 correspond to hydrogen plasma, 2 and 7 to helium plasma, 3 and 8 to argon plasma, 4 and 9 to nitrogen plasma, and 5 and 10 to oxygen plasma.

Statistical Analysis

All experiments were conducted with a minimum of two replicates. Germination parameters were assessed in two independent experiments, each with two replicated measurements. The results are presented as the mean \pm SEM and were analyzed using one-way analysis of variance (ANOVA), with significance levels indicated as (*P < 0.05, **P < 0.01, and ***P < 0.001).

Scanning Electron Microscopy

The intricate micro-morphological attributes of both untreated and plasma-treated seeds were evaluated through scanning electron microscopy (SEM). The used SEM microscope featured magnification ranges from x18 to 300,000 and an accelerating voltage range of 0.5 to 30 kV. Samples were gold-plated using the SEM Coating System at an argon pressure of 8 Pa before being examined under a

microscope. The applied gold coating was 5 nm thick. The analysis's maize samples were exposed to 30 W of plasma from each source for a total of 240 seconds.

Optical Emission Spectroscopy

Optical emission spectroscopy (OES) is an essential technique for examining plasmas with lower density and temperatures. The active plasma components were examined using optical emission spectroscopy (OES) in the case of radiofrequency (RF) plasma treatment with different gases. Since RF treatment is an indirect process, radiation had no effect on sample integrity. Five gases were used during our experiments: helium, argon, hydrogen, oxygen, and nitrogen. A small spectrometer and a fibre optic cable are required for light collection in a typical OES system. Spectral data was recorded and dispersed by an Avaspec ULS3648-USB2-VA-25 spectrometer, which examined light signals gathered by an optical fibre directed at the quartz window. This arrangement has been successfully used for plasma analysis in a treatment room.

Results

Growth Data at 30 Days

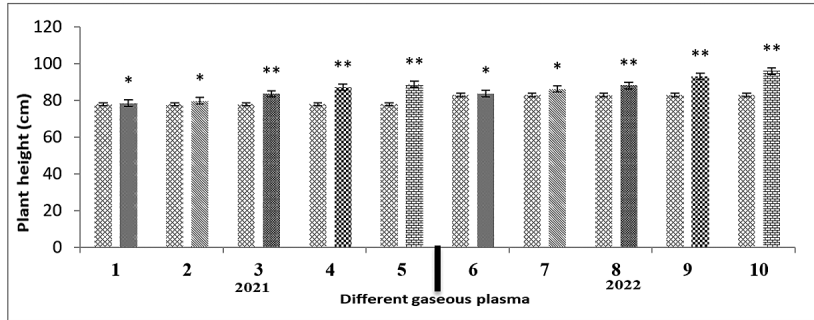
Growth parameters (plant height in cm, leaf length in cm, and plant fresh weight in g) were measured after 30 days of growth under natural field conditions in the plants that emerged from plasma treated and untreated seeds presented in Table 1. Plant height increased by 15% in the plants that emerged from the seeds treated with O₂ plasma followed by N₂ plasma (12%) (Fig. 2).

Table 1: Plant height, fresh weight of plant and leaf length of 5th leaf after 30 days of sowing plasma pre-treated seeds

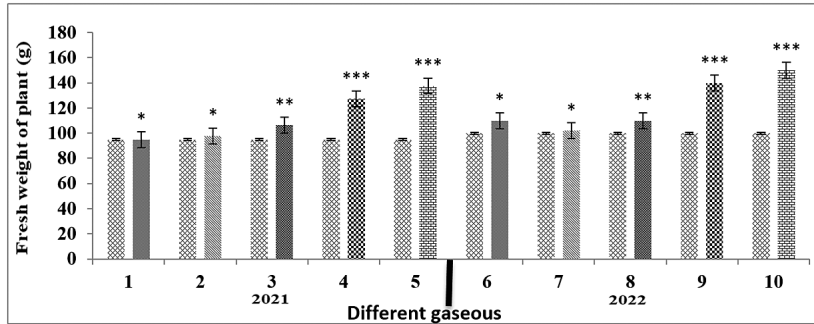
Parameters	Years	Control	H ₂ Plasma	He Plasma	Ar Plasma	N ₂ Plasma	O ₂ Plasma
Plant Height (cm)	2021	78	78.5	79.8	83.7	87.3	88.9
	2022	83	83.81	86.3	88.1	93.2	96
	Average	80.5	81.155	83.05	85.9	90.25	92.45
Fresh weight of plant (g)	2021	95	94.75	97.85	106.4	127.3	137.75
	2022	100	110	102	110	140	150
	Average	97.5	102.375	99.925	108.2	133.65	143.875
Leaf length (cm)	2021	14	14.28	14.24	14.56	17.08	17.36
	2022	16	16.3	16.5	16.5	20	20
	Average	15	15.29	15.37	15.53	18.54	18.68

The total fresh weight of the plant was increased by 48% and 37% by treatment with O₂ and N₂ plasma respectively. The maximum impact of O₂, and N₂ plasma treatment was in the enhancement of leaf

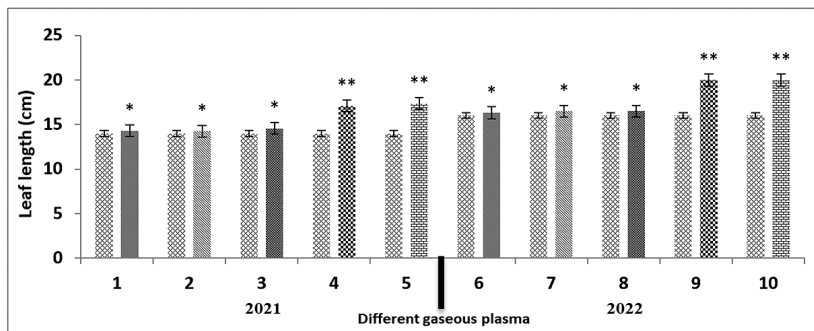
length which showed an enhancement of 24% and 25% (Fig. 2). The impact of plasma treatment with other gases viz. H₂, He and Ar, on the growth parameters were much lesser (Fig. 2).



(a)



(b)



(c)

Fig. 2: Plant height (a), fresh weight of plant (b) and leaf length (c) of 5th leaf after 30 days of sowing plasma pre-treated seeds. Vertical line above the bar indicates \pm SEM. *, ** and * indicate significance at $P < 0.001$, 0.01 , and 0.05 , respectively, compared to control.**

The Growth Data at 60 Days

The enhancement in the growth of the field grown plants showed a similar trend as seen at 30 days of growth shows in Table 2. Maximum enhancement in

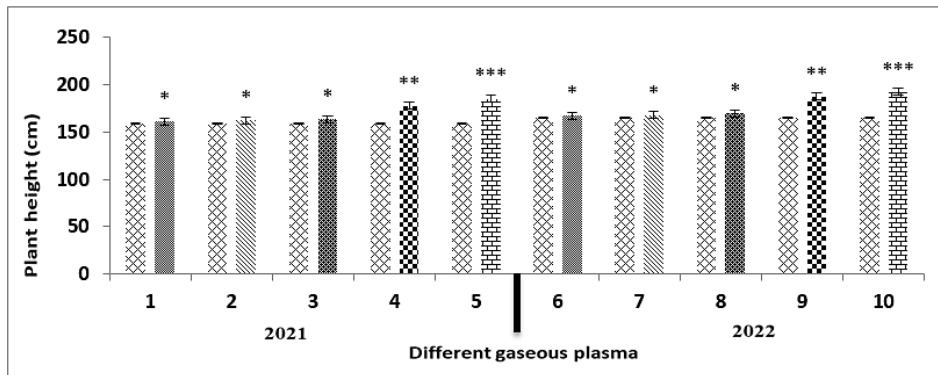
the plant height, fresh weight and leaf length were recorded in plants that emerged from seeds treated with O₂ plasma followed by N₂ plasma (Fig. 3). Plants that emerged from seeds treated with Ar plasma showed better enhancement compared to H₂ and He

plasma (Fig. 3). At 60 days of growth cobs formed on the plants that emerged from the seeds treated with O₂, N₂ and Ar plasma had more weight compared to the cobs formed on the untreated control plants

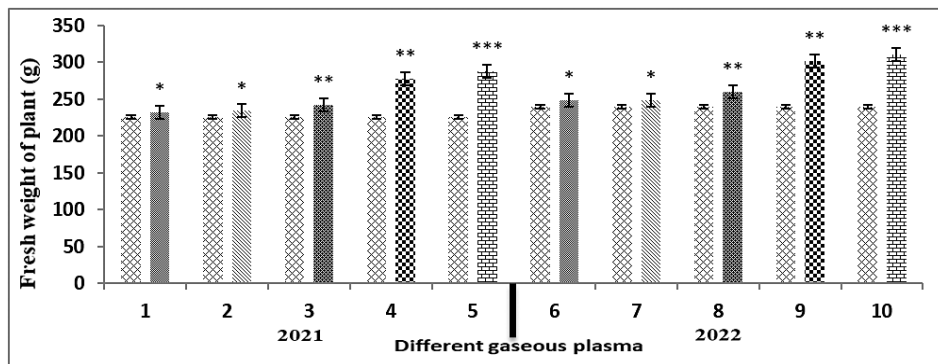
(Fig. 3). Treatment of seeds with H₂ and He plasma reduced the weight of the cobs formed at 60 days of growth (Fig.3).

Table 2: Plant height, fresh weight of plant and leaf length of 5th leaf and cob weight after 60 days of sowing plasma pre-treated seeds

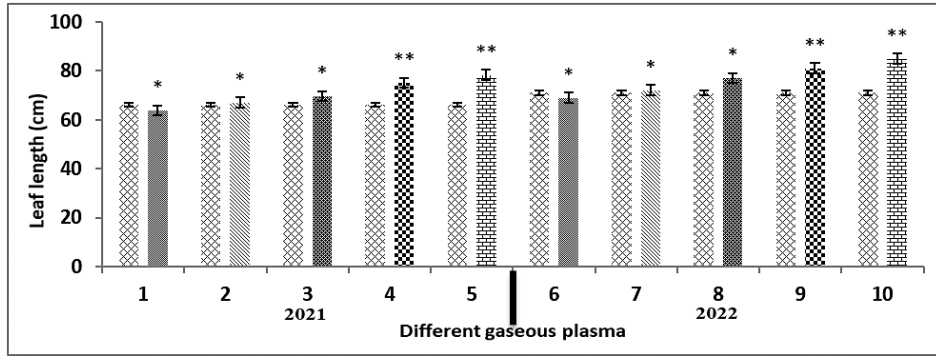
Parameters	Years	Control	H ₂ Plasma	He Plasma	Ar Plasma	N ₂ Plasma	O ₂ Plasma
Plant Height (cm)	2021	159	160.91	162.2	163.6	177.8	185.25
	2022	165	167.3	168	169.7	187.5	192.5
	Average	162	164.105	165.1	166.65	182.65	188.875
Fresh weight of plant (g)	2021	225	231.75	234	241.9	277.9	288
	2022	240	248	248.4	260	301.5	310.5
	Average	232.5	239.875	241.2	250.95	289.7	299.25
Leaf length (cm)	2021	66	63.76	67.06	69.6	75.11	78.3
	2022	71	69	72.1	77	81	85
	Average	68.5	66.38	69.58	73.3	78.055	81.65
Cob weight (g)	2021	68	59.4	61.2	80.9	87.2	92.5
	2022	70	59	62.6	83	91	95
	Average	69	59.2	61.9	81.95	89.1	93.75



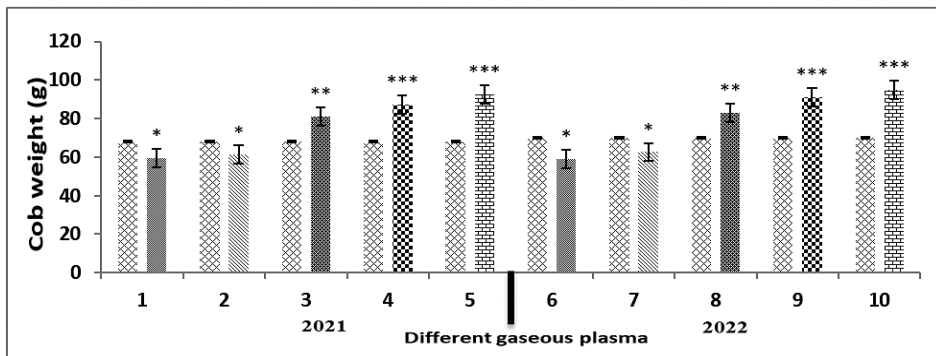
(a)



(b)



(c)



(d)

Fig. 3: Plant height (a), fresh weight of plant (b), leaf length (c) and cob weight (d) after 60 days of sowing plasma pre-treated seeds. Vertical line above the bar indicates \pm SEM. ***, ** and * indicate significance at $P < 0.001$, 0.01 , and 0.05 , respectively, compared to control.

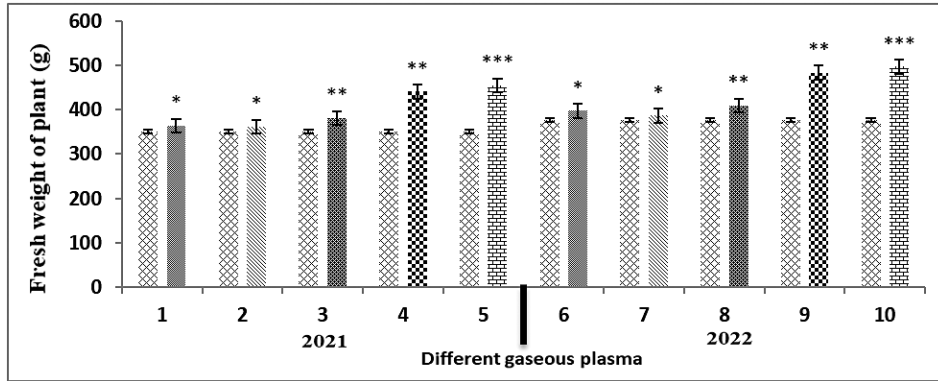
Table 3: Fresh weight, cob length, cob weight, and 100 seed weight after 90 days of sowing of plasma pre-treated seeds

Parameters	Years	Control	H ₂ Plasma	He Plasma	Ar Plasma	N ₂ Plasma	O ₂ Plasma
Fresh weight of plant (g)	2021	350	363.65	361.2	381.5	441	455
	2022	376	397.6	387	409	484	497.5
	Average	363	380.625	374.1	395.25	462.5	476.25
Length of cob (cm)	2021	25.6	25.9	25.8	27.65	30.2	31
	2022	27.8	28	28	30	33.2	33.6
	Average	26.7	26.95	26.9	28.825	31.7	32.3
Cob weight (g)	2021	172	178	180.25	182.32	227.9	231.7
	2022	186	192	194	200	252	257
	Average	179	185	187.125	191.16	239.95	244.35
100 seed fresh weight (g)	2021	18	18.27	18.36	18.61	24.25	24.86
	2022	20.5	21	20.73	21.4	28.2	28.9
	Average	19.25	19.635	19.545	20.005	26.225	26.88

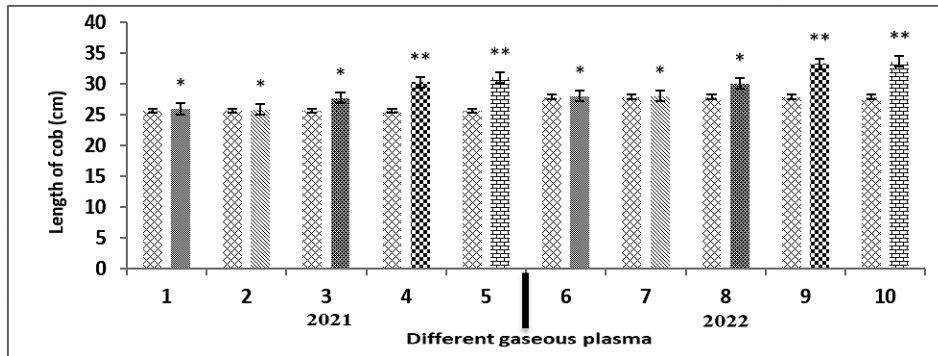
Yield Data at 90 Days

Table 3 presents the yield data, Maize plants attained maturity at 90 days and the yield data was measured in terms of plant fresh weight in g, cob length in cm, cob weight in g and 100 seeds weight in g (Fig. 4). O₂ and N₂ plasma treated seeds showed maximum enhancement in the yield. O₂ plasma treatment of

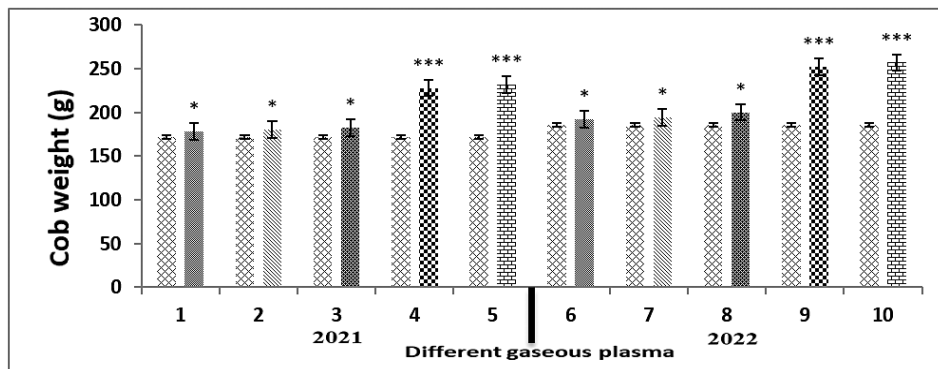
seeds enhanced the plant weight by 31%; cob length by 21%, cob weight by 36.50% and 100 seeds weight by 39% (Fig. 4). The impact of N₂ plasma treatment on the yield of seeds was very close to that of O₂ plasma (Fig. 4). Of the other gases, Ar was more effective than H₂ and He (Fig. 4).



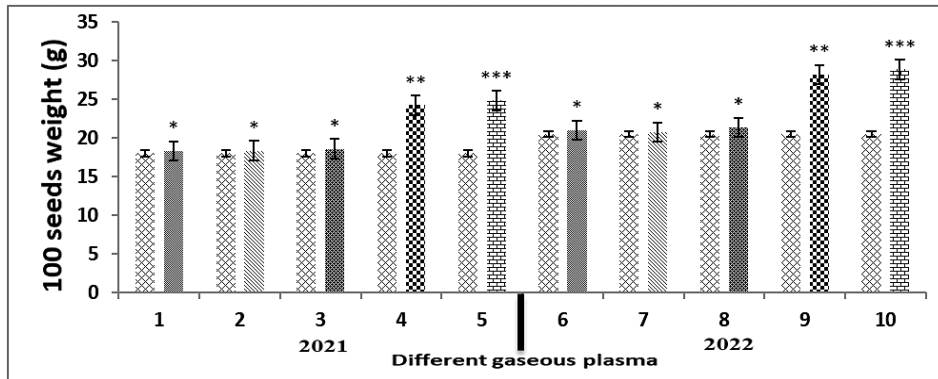
(a)



(b)



(c)



(d)

Fig. 4: Fresh weight (a), cob length (b), cob weight (c) and 100 seed weight (d) after 90 days of sowing of plasma pre-treated seeds. Vertical line above the bar indicates \pm SEM. ***, ** and * indicate significance at $P < 0.001$, 0.01 , and 0.05 , respectively, compared to control.

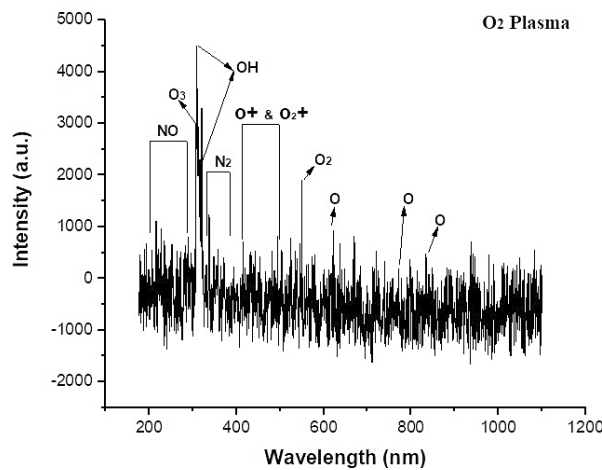
SEM

Using a scanning electron microscope, the control, and plasma-treated seeds were compared to learn more about the changes in seed surface topology, structure, and general surface properties. SEM analysis was commonly employed to investigate alterations in the morphology of seed coats.¹⁴ crack-like damage in cabbage seeds following treatment with low-pressure O₂ and air plasma, attributing these cracks to factors such as oxidation, collision of active species generated by the plasma¹⁵ or thermal expansion through plasma treatment. Studied wheat seed coats before and after DBD plasma treatment, noting a shift from a clear boundary

layer to a softer structure with indistinct boundaries and crack formations post-treatment. Wheat seed cross-sections exhibited a netlike formation in the endosperm before plasma treatment, with a clear release of starch grains and increased availability of free starch after treatment.¹⁶

OES

The graph below shows the optical emission spectra of the control, oxygen, nitrogen, argon, helium, and hydrogen plasmas. The optical emission spectrum is one quick, real-time, and non-destructive way for analyzing the radicals, active species, atoms, and ions created in plasma.¹⁷



(a)

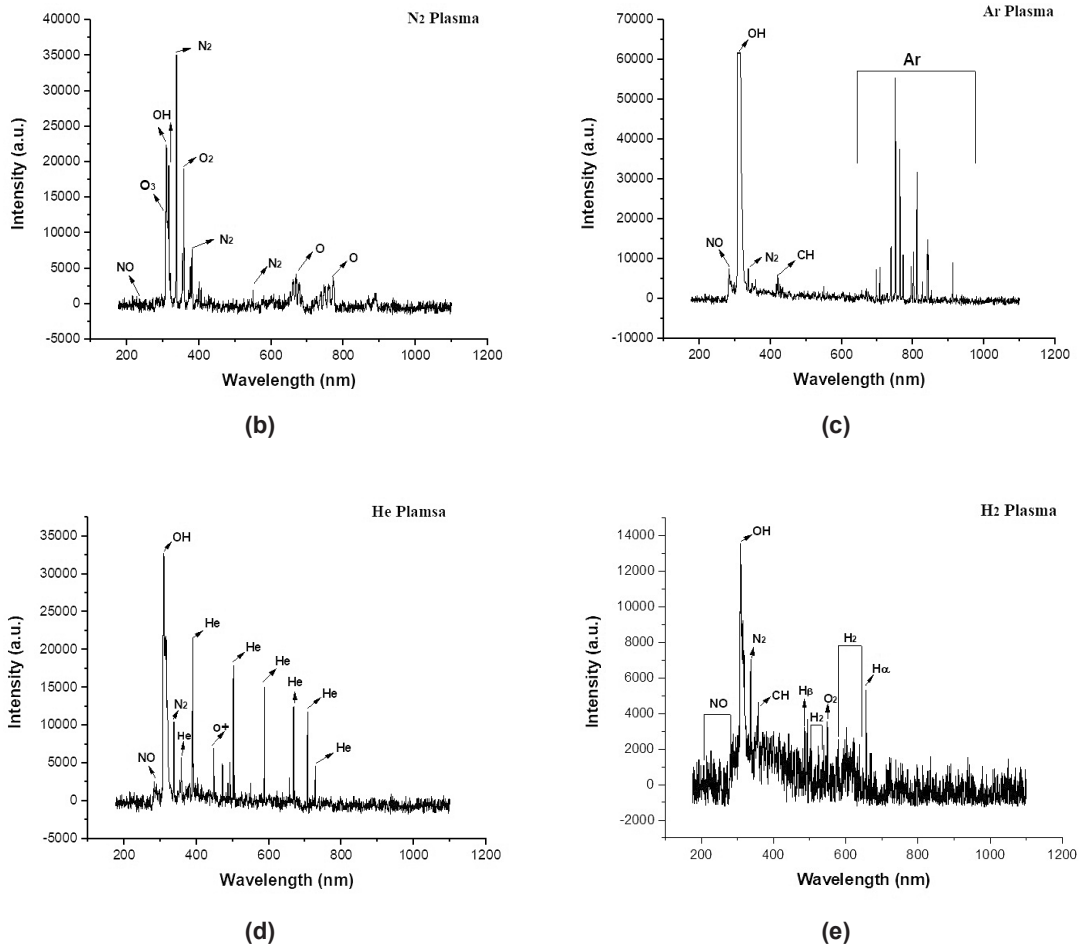


Fig. 5: OES of Oxygen (a), Nitrogen (b), Argon (c), Helium (d), and Hydrogen (e) plasma pre-treated seeds

Figure 5 uses OES to display the RONS produced during plasma in the gaseous phase for different types of gases. During the O₂ plasma, we found a strong OH peak at 306.9 nm with high intensity, a small NO peak at 200–280 nm, and the O₂ 1st positive system at 777.2 and 844.1 nm, respectively. For N₂ plasma, we detected the OH peak at 306.9 nm and 309 nm, the greatest peaks at 358 nm, and also observed the N₂ peaks in the range (500–700 nm). Moreover, for Ar plasma, we observed the biggest OH peak at 309.6 nm, and additional Ar lines in the range (650–850) nm. For the Air plasma, we observed small emission lines in the range (200–250) nm that belong to the molecular NO β, γ system. Strong peaks were detected for N₂ at 350–380 nm, and CH peaks were detected at 420 nm. In helium plasma, a strong peak

of OH at 309 nm with high intensity was detected, a major NO peak was detected at 280 to 290 nm, and a He peak at 388 nm, 502 nm, 588 nm, 667 nm, and 706 nm. In hydrogen plasma, we observed the biggest OH peak at 309 nm, and small NO peaks from 200–300 nm, we found the H_α and H_β at 656 nm and 486 nm, and also H₂ peaks were observed between 580 to 650 nm. The sterilization process was significantly aided by these reactive oxygen and nitrogen species (RONS).

Discussion

Although several studies have been done on germination and growth of seedlings after the pre-treatment of seeds with plasma, very few studies have been done to record the impact of

pre-treatment of seeds with plasma on the yield of crop plants at the field level. Enhancement in germination and productivity of *Lupinus angustifolius*, *Galega virginiana*; *Melilotus albus*,¹² *Lycopersicum esculentum*,¹¹ corn¹³ A by using plasma Pre-sowing treatment of seeds have been reported. In corn,¹³ used low-pressure RF N₂ plasma and DBD He plasma to study the enhancement in productivity at the field level.

In the present paper, data has been obtained for two years, 2021 and 2022 (July to October) at field level on the growth and yield of maize (*Zea mays* var: GSF2) after Pre-sowing treatment of seeds with plasma using five different gases (O₂, N₂, H₂, He and Ar). Of the five gases used, O₂ and N₂ plasma are the most effective in promoting the growth and yield of maize under field conditions. The consistency in weather conditions over the two years likely played significant role in the stability of the observed results. When environmental conditions such as temperature and rainfall remain relatively stable, plants experience uniform growth and development patterns. Plasma treatment offers practical benefits by enhancing growth and yield, reducing the demand for agricultural chemicals, and enhancing disease resistance. Plasma treatment does not produce any chemical or radical that is not naturally produced in the plant tissues. Plasma treatment at best changes the expression of genes involved in germination and other biological processes 14 using the free radicals produced.

Enhanced growth of the leaves in terms of area after Pre-sowing treatment of seeds with plasma contributes to enhanced rate of photosynthesis and yield. Leaf length was enhanced by 24% and 25% at 30 days by O₂ and N₂ plasma (Fig. 2) and this difference was reduced to 19% (O₂) and 14% (N₂) at 60 days (Fig. 3). Reduction of difference between leaf area of plants that emerged from untreated and plasma treated seeds at 60 days of growth indicates that the leaf growth is faster during the early vegetative stage after treatment with plasma. Pre-sowing treatment of seeds with He, H₂ and Ar plasma also enhances growth and yield of maize, albeit marginally. Argon plasma enhances weight of the cobs by 7% and seeds weight by 4% (Fig.4). The enhancement in growth and yield by using He and H₂ is negligible.

At maturity the cob weight was enhanced by 36.50% in O₂ and 34% in N₂ plasma (Fig. 4). Along with this enhancement the seeds were bold and 100 seeds weight was enhanced by 39% in O₂ and 36% in N₂ plasma (Fig. 4). An increase in the total weight of the plant of 31% in O₂ and 27% in N₂ plasma and length of cob was 21% in O₂ and 19% in N₂ plasma indicates the additional advantage of using plasma which can enhance biomass that is utilized as fodder. The coefficient of variations for 100 seeds weight was 6.49% in control, 7.52% in O₂ plasma and 7.53% in N₂ plasma. Our data indicates that O₂ and N₂ plasma are most effective for the pre-sowing treatment of maize seeds and will be significantly beneficial at the field level to increase the yield.

SEM analysis indicated significant modifications in oxygen, nitrogen, argon, and helium, with no discernible impact on hydrogen plasma treatment. The results suggest that the surface of the starch caryopsis underwent alterations, exposing oxygen and nitrogen plasma, resulting in substantial channels and water holes.

OES identified the presence of OH, NO, O, and N₂ radicals during oxygen and nitrogen plasma treatment, contributing to the most favorable outcome (Fig. 5). This chemical-free approach has the potential to address global food shortages.

Conclusion

In conclusion, we have examined maize crop data for two consecutive years, 2021 and 2022 (July to October) and they show consistent results. Additionally, there were consistent weather conditions throughout the growth of the maize crop during this period. Our data indicates that O₂ and N₂ plasma are most effective for the pre-sowing treatment of maize seeds and will be significantly beneficial at the field level to increase the yield. For maize seed the increase in yield is more in case O₂ in compare to control and others gases used. Experiments are being continued in our laboratory to understand the metabolic changes induced by O₂ and N₂ plasma in maize seeds. SEM analysis showed significant changes for argon, oxygen, nitrogen, and helium but no change for hydrogen plasma treatment. The results show that the starch caryopsis' surface has been modified, revealing an oxygen and nitrogen plasma that has produced vast channels and water holes. The greatest outcome was achieved after

oxygen and nitrogen plasma treatment, as shown by the OES, which also showed the existence of OH, NO, O, and N₂ radicals. Therefore, our findings imply that oxygen and nitrogen glow discharge plasma treatment has a favorable impact on the maize plant and its output, which could be exploited in the future to expand agronomic opportunities.

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The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author's Contribution

- **Kundan Viliya:** Writer, Resources, Conceptualization, Data Curation
- **Uttam Sharma:** Supervision, Writing -Review and Editing
- **Manisha Thakur:** Formal analysis
- **Guruprasad Kadur Narayan:** Supervision, Writing- Review and Editing
- **Jayshree Sharma:** Resources
- **Ramkrishna Rane:** Resources
- **Amulya sanyasi:** Resources
- **Joydeep Ghosh:** Visualization, Supervision and Writing- Review and Editing

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