ENHANCEMENT OF SOYBEAN (*GLYCINE MAX* VAR. *JS-9560*) GROWTH AND YIELD AFTER PRE-SOWING TREATMENT OF SEEDS USING NON-THERMAL PLASMA OF DIFFERENT GASES

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Abstract. Pre-sowing treatment of seeds with non-thermal plasma of different gases has been studied on the growth and yield of soybean (*Glycine max* var. *JS-9560*) under field conditions for two consecutive years (2022 and 2023). Five types of gases, N_2, He, Ar, H_2, and O_2 were used to create plasma in a cylindrical vacuum container with an RF power supply. Soybean seeds were exposed to plasma for 15 seconds at 80 W. Growth measurements were taken at 30, 60, and 90 days after sowing the seeds. Plasma treatment improved germination, plant height, leaf area, and plant fresh weight at all the stages of the vegetative growth period. N_2 and He plasma were most effective in promoting growth and biomass compared to Ar, O_2, and H_2. The yield parameters in terms of the number of pods, number of seeds, and 100 seed weight were improved to the extent of around 60 % by N_2 and He plasma. Other gases (Ar, H_2, and O_2) were less beneficial in increasing the yield. Pre-sowing treatment of seeds with plasma especially with N_2 and He would be highly beneficial for enhancing the yield of soybean at the field level. The characterization of soybean seeds was conducted using scanning electron microscopy (SEM), Fourier transform infrared (FTIR), and optical emission spectroscopy (OES). SEM analysis showed significant changes in nitrogen, helium, argon, oxygen, and hydrogen plasma treated seeds, indicating alterations on the surface of the starch caryopsis, resulting in large channels and pores. FTIR spectroscopy indicated modifications after exposure to plasma-treated seeds, suggesting surface activation due to lipid breakdown. OES detected OH, NO, O, and N_2 radicals during plasma treatment, contributing to the optimal outcome

Key words: N_2, He, Ar, O_2, H_2 plasma, soybean, germination, growth, yield, SEM, FTIR, OES.

INTRODUCTION

In the modern agriculture, there have been attempts to employ techniques that are environmental friendly and contribute significantly towards increasing the yield of

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crops. One of the physical methods used to improve the yield of crops is the pre-sowing treatment of seeds with low magnetic fields. On soybean (*Glycine max* L.) pre-treatment of seeds with a pulsed magnetic field enhanced the growth and yield at the field level [16]. Earlier to this had reported enhancement of photosynthesis in soybean by pre-treatment of seeds with magnetic field [20]. Similarly cold plasma treatment offers a swift, cost-effective, and environmentally friendly approach to enhance seed performance and boost crop yield [8, 11]. It plays crucial roles across various plant developmental and physiological processes. These include diminishing seed bacterial contamination, modifying seed coat compositions, enhancing seed coat permeability, and promoting seed germination as well as seedling growth [6, 13, 25]. Treatment of soybean seeds with cold plasma enhanced seed germination and seedling growth in the Chinese variety (*Glycine max* cv. *Zhongdou 40*) [21]. In the present paper, a detailed investigation was conducted on the growth and yield of soybean (*Glycine max* cv. *9560*) at the field level. The study covered two consecutive years, 2022 and 2023. Five different types of plasma were used in the investigation. The plasmas were produced with O_2, N_2, He, Ar, and H_2. The majority of the studies conducted earlier on the pre-sowing treatment of seeds with plasma have reported enhanced seed germination and seedling growth in crops like wheat, corn, soybean, tomato, etc. The initial effects of plasma on the seeds seem to be due to the reactive free radicals of oxygen like superoxide (O_2^-), hydroxyl (OH), and nitric oxide (NO) radicals, all of which have bactericidal effects and are capable of decontamination of seed surface [5]. It plays crucial roles in a diverse array of developmental and physiological processes within plants. This occurrence has been observed in various plant species, including *Chenopodium album* [19], *Oryza sativa* [3], *Triticum aestivum* [18], *Lycopersicon esculentum* [12], and *Solanum melongena* L. [23]. Furthermore, plasma treatment has been shown to enhance the plant's physiological metabolism, such as increasing dehydrogenase activity, superoxide dismutase, and peroxidase activities [10], as well as improving photosynthetic pigments, photosynthetic efficiency, and nitrate reductase activity [24]. In addition to this, plasma exposure also has a physical effect on the outer layers of the seed which results in the enhancement of water uptake [5]. Concerning crop yield, enhancement in the yield of tomato and corn by pre-sowing treatment of seeds with plasma has been reported not many crops have been tested at the field level for analyzing the impact of the pre-sowing treatment of seeds with plasma on the growth and yield of crops [9, 17]. The present investigation therefore has the objective of studying the plasma produced with five different gases on the growth and yield of an Indian variety of soybean.

MATERIAL AND METHODS

Seeds of soybean (*Glycine max* var. *JS-9560*) were acquired from Ms. Jain Seed Agency in Indore, Madhya Pradesh, India. A careful selection of healthy and consistent seeds was done for the pre-sowing treatment with plasma.

STUDY AREA OF THE LOCATION

This study was conducted in the agricultural field of “Shri Vaishnav” Institute of Agriculture Science (SVVV) in Indore during the consecutive cropping seasons of 2022 and 2023. The research site is located at latitude of 22.82 and a longitude of 75.84. Data collection occurred from July to October, covering the cropping seasons in India.

PLASMA TREATMENT

A system for producing glow-discharge plasma utilizing RF and DC power supplies assembled in the Plasma Research Laboratory was used for the pre-sowing treatment of seeds. The system consists of a cylindrical stainless steel vacuum chamber (SS304; height 30 cm; diameter 36 cm; volume 33 liters) fitted with cathode assembly electrode. A RF power source of 13.56 MHz; 600W and an automated matching network are employed for RF electric gas discharge. A rotary pump within the reactor generates a vacuum of 1×10^{-3} mbar. Various types of gases are supplied to the chamber by using a rotating pump.

Fig. 1. Vacuum chamber (a), seeds holder (b).

In Figure1 seeds were placed in the vacuum chamber between the electrodes on a pedestal with the help of a specially designed seed holder. Hundred seeds were placed on the seed holder for each exposure to plasma. After placing the seeds inside the chamber a vacuum of 4×10^{-2} mbar was created with the help of the rotary pump. Once the vacuum reached 2×10^{-1} mbar, one of the gases (nitrogen, helium, argon, hydrogen, or oxygen) was injected into the chamber through a dosing valve at a flow rate of 100 mbar L/s before applying RF power to the electrodes. Seeds were treated with plasma for 15 seconds at 80 W. The energy level and the time of exposure were standardized dose response curves obtained by varying energy levels and the time of exposure. Following treatment, the gas flow was continued for an additional five minutes to get rid of any unwanted reactive compounds. The treated seeds were taken out of the chamber and stored in sealed plastic bags for use in the fields sowing and growth and yield in the field experiments were carried out in the agricultural field of "Shri Vaishnav" Institute of Agriculture Science. Initially, we thoroughly plowed the field and made thorough preparations for planting soybean. Our crop is dependent on the rainy season. The sowing of untreated or plasma-treated seeds was conducted using a randomized block design. Each seed row spanned 20 meters with a 50 cm row-to-row distance and a 15 cm seed-to-seed spacing. Across a total of 18 rows, three rows were allocated for untreated control seeds, while the remaining 15 rows were dedicated to sowing seeds treated with plasma generated from five different gases (N_2, He, Ar, H_2, O_2), utilizing three rows for each gas. In both 2022 and 2023, we planted 100 seeds in the field, including untreated seeds and those treated with plasma using different gases. Throughout both years 2022 and 2023 of the crop season, the overall precipitation ranged between 40 and 45 inches. The highest germination rates were observed with nitrogen compared to untreated seeds, followed by helium, argon, oxygen and hydrogen. Growth measurements were taken at 30, 60, and 90 days post-sowing. The presented growth data represents the average of five plants, while yield data reflects the average of ten plants. The field data represents the mean percentages from both 2022 and 2023. For a more intricate analysis, detailed information is presented in Tables 1, 2, and 3 as well as Figures 2, 4, 6, and 8.

SCANNING ELECTRON MICROSCOPY

The intricate micro-morphological attributes of both untreated and plasma-treated seeds were evaluated through scanning electron microscopy (SEM). The SEM microscope employed had an accelerating voltage ranging from 0.5 to 30 kV and offered magnification levels from ×18 to 300,000. Prior to microscopy, samples underwent gold plating using the SEM coating system under argon pressure of 8 Pa. The gold layer applied had a thickness of 5 nm. Soybean samples subjected to this analysis were treated by each plasma source at 80 W for duration of 15 seconds.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

Fourier transform infrared spectroscopy (FTIR) examination of the soybean seeds was conducted utilizing the SHIMADZU 8900 FTIR. Through (FTIR), it was revealed that certain chemical bonds underwent alterations following exposure to plasma using different operational gases on soybean seeds. The spectrometer facilitated the measurements, with spectra acquired using 20 scans for both background and sample, at a resolution of 2 (1/cm), within the range of 4000–400 1/cm. The seeds were ground to make powder and FTIR was performed in transmission mode.

OPTICAL EMISSION SPECTROSCOPY

Optical emission spectroscopy (OES) serves as a vital method for scrutinizing plasmas characterized by lower temperatures and densities [26]. In the case of radio frequency (RF) plasma treatment with various gases, optical OES was utilized to examine the active plasma constituents. Given the indirect nature of RF treatment, sample integrity remained unaffected by radiation. Our experimentation involved the application of five gases: nitrogen, oxygen, hydrogen, argon, and helium. A standard OES setup comprises a fiber optic cable for light capture and a compact spectrometer. Light signals were collected via an optical fiber aimed at the quartz window and then analyzed by an Avaspec ULS3648-USB2-VA-25 spectrometer, responsible for dispersing and recording spectral data. This configuration has been effectively deployed for plasma analysis within a treatment chamber.

RESULTS

The laboratory and field data have been presented for 2022 and 2023 in the results. In the graph, the mean of two years of data is given in the table.

GROWTH DATA AT 30 DAYS

Growth parameters, including plant height, plant fresh weight, and leaf area, were assessed after 30 days of growth under field conditions for plants originating from both plasma-treated and untreated seeds. Plant height exhibited a 48 % increase in plants derived from He plasma-treated seeds, followed by Ar plasma (34 %), O_2 (22 %), N_2 (26 %), and H_2 plasma (17 %) (Figs. 2 and 3). The total plant fresh weight experienced a 49 % increase with He plasma treatment, followed by N_2 plasma (14 %), H_2 plasma (9 %), Ar plasma (18 %), and O_2 (11 %) (Fig. 3). The maximum impact of He, O_2, and N_2 plasma treatments was observed in the enhancement of leaf area, showing increases of 44 %, 25 %, and 25 %, respectively, while H_2 plasma (14 %) and Ar plasma (20 %) also exhibited notable impacts. The overall effect of plasma treatment with all gases, including He, N_2, H_2, O_2, and Ar, on growth parameters was significantly greater (Figs. 2 and 3).

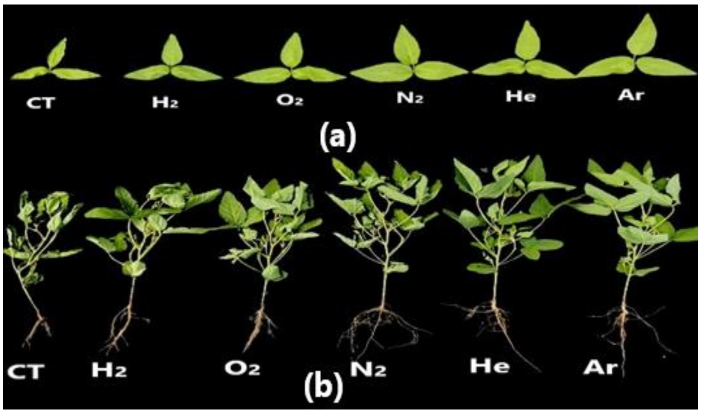
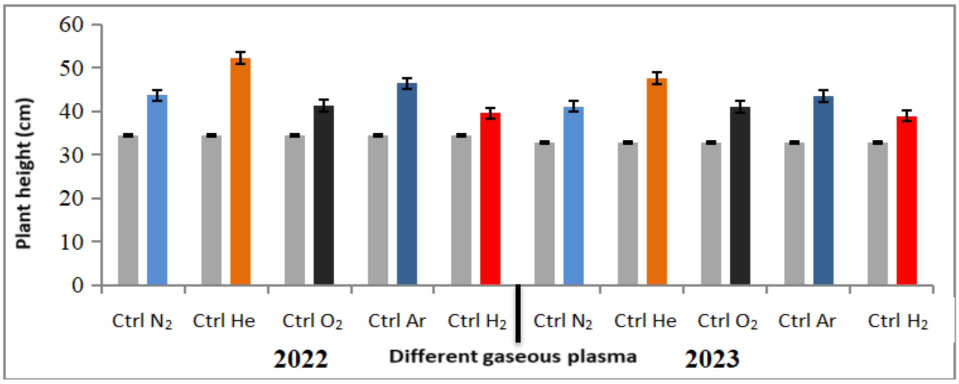


Fig. 2. Leaf area (a) and plant height (b) after 30 days of sowing of plasma pre-treated seeds.

Table 1

Plant height, fresh weight of plant and leaf area after 30 days of sowing plasma pre-treated seeds

Parameters	**Years**	**Control**	**N_2 plasma**	**He plasma**	**O_2 plasma**	**Ar plasma**	**H_2 plasma**
Plant height (cm)	2022	34.4	43.7	52.3	41.3	46.44	39.6
	2023	32.8	41.2	47.7	41	43.6	39
	mean	**33.6**	**42.45**	**50**	**41.15**	**45.02**	**39.3**
Fresh weight of plant (g)	2022	7.5	8.43	10.53	8.26	9.38	7.8
	2023	7.7	8.9	12.11	8.54	8.6	8.7
	mean	**7.6**	**8.665**	**11.32**	**8.4**	**8.99**	**8.25**
Leaf area (cm^2)	2022	17.3	21.8	24.57	20.9	22	18.68
	2023	20.5	25.5	30	26.5	23.5	24.5
	mean	**18.9**	**23.65**	**27.285**	**23.7**	**22.75**	**21.59**



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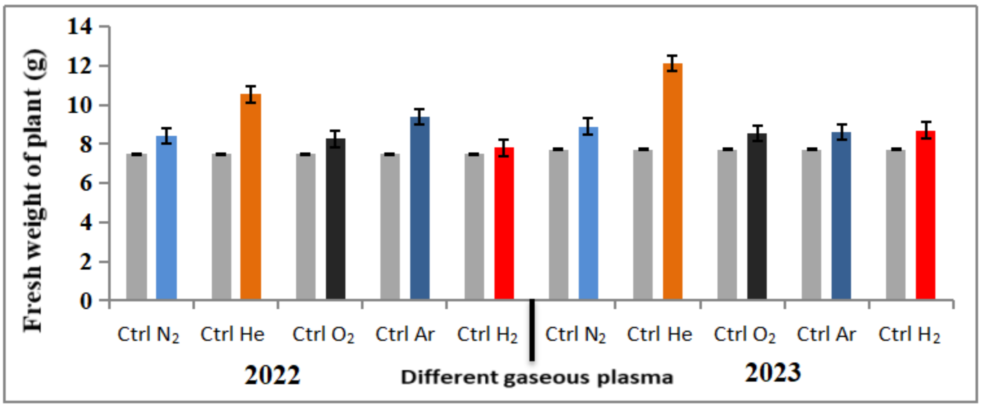

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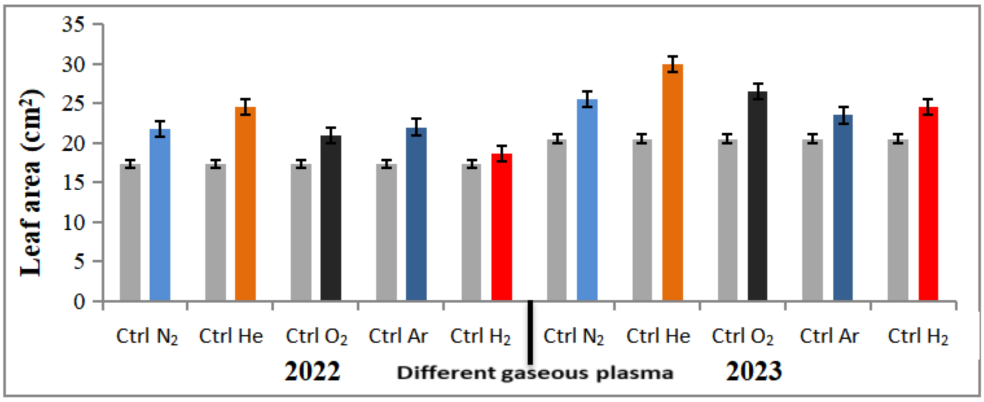


(c)

Fig. 3. Plant height (a), fresh weight (b), and leaf area (c) after 30 days of sowing of plasma pre-treated seeds.

GROWTH DATA AT 60 DAYS

The improvement in the growth of field-grown plants exhibited a consistent pattern as observed at 30 days of growth. The most substantial enhancements in plant height, fresh weight, and leaf area were observed in plants arising from seeds treated with He plasma, followed by Ar plasma (Figs. 2 and 3). Plants from seeds treated with N_2 plasma demonstrated superior enhancement compared to those treated with H_2 and He plasma (Figs 4 and 5). By the 60th day of growth, plants that emerged from seeds treated with He, N_2, Ar, and O_2 plasma displayed a greater number of pods compared to untreated control plants (Fig. 4). Treatment with H_2 plasma had a relatively minor effect on the number of pods formed at the 60-day mark (Fig. 4).

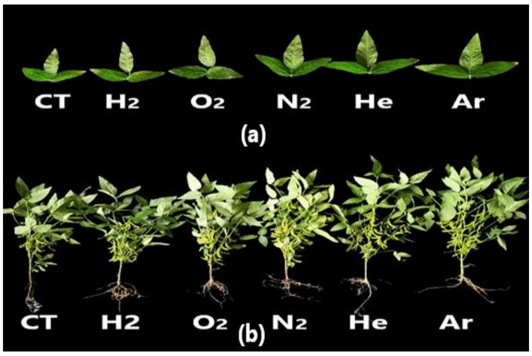
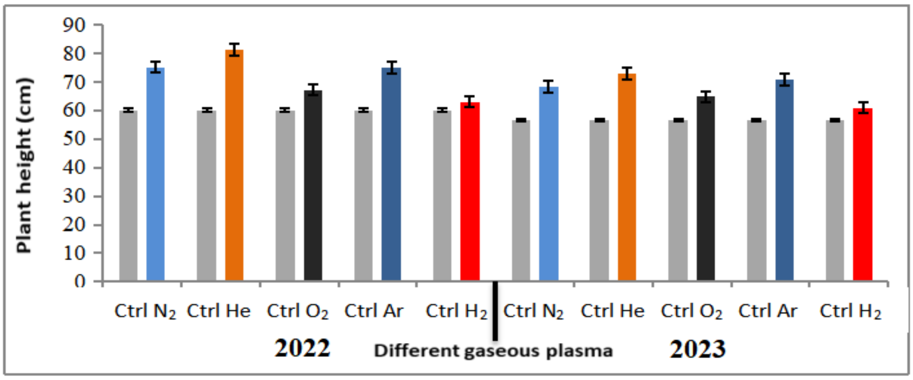


Fig. 4. Leaf area (a) and plant height with pods (b) after 60 days of sowing of plasma pre-treated seeds.

Table 2

Plant height, fresh weight of plant and no. of pods/plant after 60 days of sowing plasma pre-treated seeds

Parameters	Years	Control	N_2 plasma	He plasma	O_2 plasma	Ar plasma	H_2 plasma
Plant height (cm)	2022	60	75.1	81.2	67.2	75.06	63
	2023	56.64	68.32	72.89	64.77	70.86	60.96
	mean	**58.32**	**71.71**	**77.045**	**65.985**	**72.96**	**61.98**
Fresh weight of plant (g)	2022	320	600	540	480	500	420
	2023	350	700	650	600	650	500
	mean	**335**	**650**	**595**	**540**	**575**	**460**
Leaf area (cm^2)	2022	28	35.3	37	33.6	36.4	29.06
	2023	30.5	39.75	41.5	37.5	39	32.8
	mean	**29.25**	**37.525**	**39.25**	**35.55**	**37.7**	**30.93**
No. of pods/plant	2022	35	64	58	50	55	42
	2023	38	72	64	60	62	48
	mean	**36.5**	**68**	**61**	**55**	**58.5**	**45**



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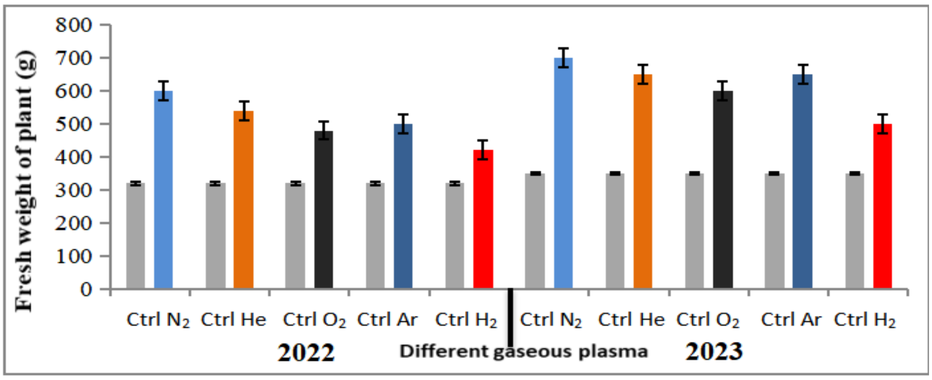

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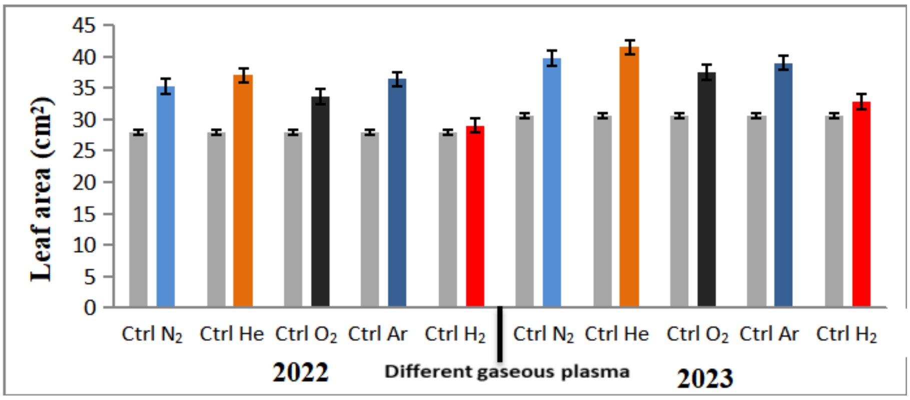

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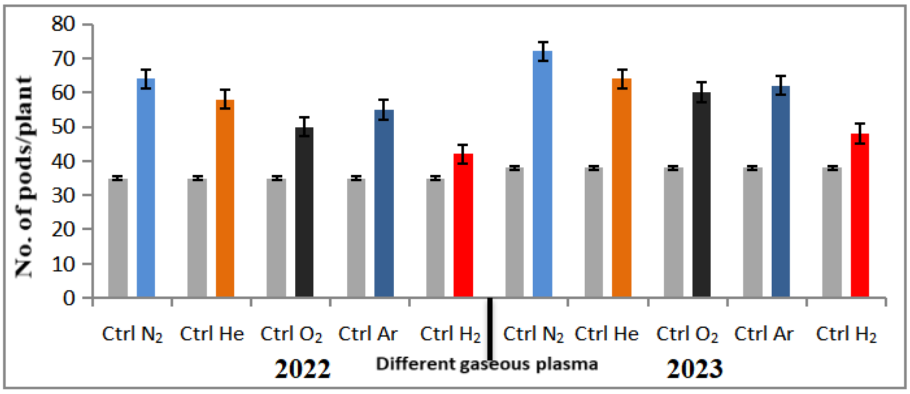


(d)

Fig. 5. Plant height (a), fresh weight (b), leaf area (c), and no. of pods/plant (d) after 60 days of sowing of plasma pre-treated seeds.

YIELD DATA AT 90 DAYS

Soybean plants reached maturity at 90 days, and the yield data were assessed in terms of plant fresh weight, number of pods, number of seeds, and 100 seeds weight (Figs. 6 and 7). Seeds treated with N_2 and He plasma exhibited the most significant improvements in yield. Specifically, N_2 plasma treatment increased plant weight by 60 %, the number of pods by 69 %, the number of seeds by 61 %, and the 100-seed weight by 64 % (Fig. 6). The impact of O_2, Ar, and H_2 plasma treatments on seed yield was notably favorable compared to the untreated control (Fig. 6). Among the other gases, O_2 proved more effective than Ar and H_2, but for 100-seed weight, Ar was more effective than O_2 (Fig. 6).

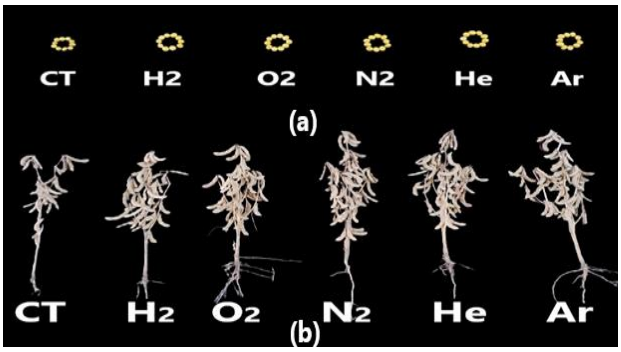
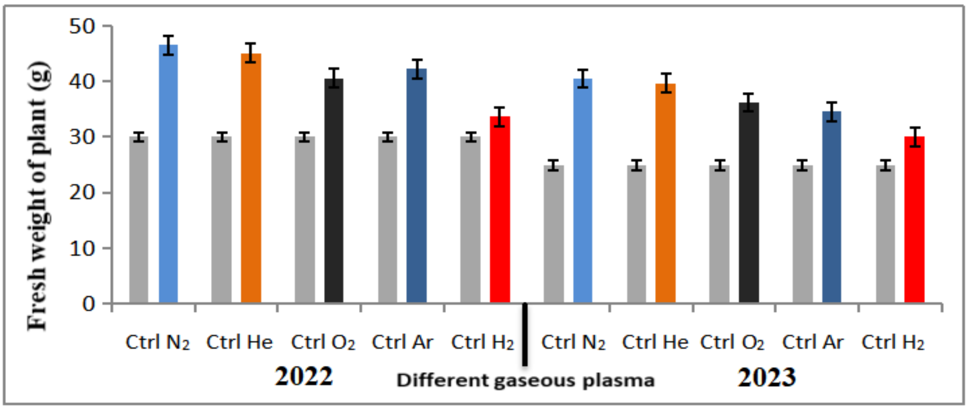


Fig. 6. Seeds (a), plant height after 90 days of sowing of plasma pre-treated seeds.

Table 3

Fresh weight, no. of pods/plant, no. of seeds/plant, and 100 seed weight after 90 days of sowing of plasma pre-treated seeds

Parameters	**Years**	**Control**	**N_2 plasma**	**He plasma**	**O_2 plasma**	**Ar plasma**	**H_2 plasma**
Fresh weight of plant (g)	2022	30	46.5	45.1	40.6	42.2	33.6
	2023	24.84	40.48	39.65	36.16	34.5	30
	mean	27.42	43.49	42.375	38.38	38.35	31.8
No. of pods/plant	2022	40	66	60	52	56	45
	2023	37	64	57	54	50	53
	mean	38.5	65	58.5	53	53	49
No. of seeds/plant	2022	101	155	142	138	135	115
	2023	97	164	148	150	133	118
	mean	99	159.5	145	144	134	116.5
100 seed fresh weight (g)	2022	12	18.6	16	15.6	15.9	13
	2023	10	17.4	16	15.5	15.8	12.4
	mean	11	18	16	15.55	15.85	12.7


Fresh weight of plant (g)
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Different gaseous plasma
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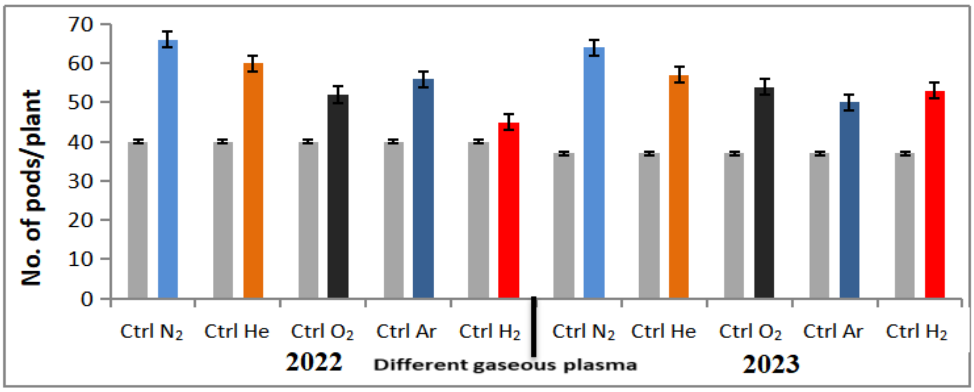
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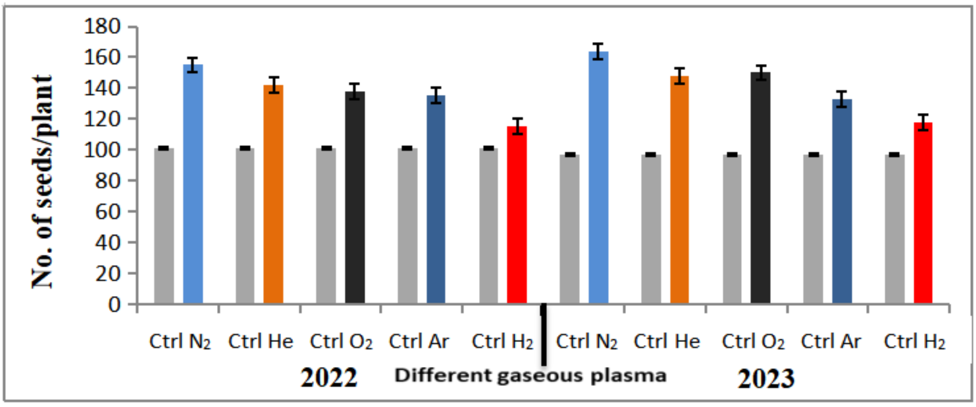
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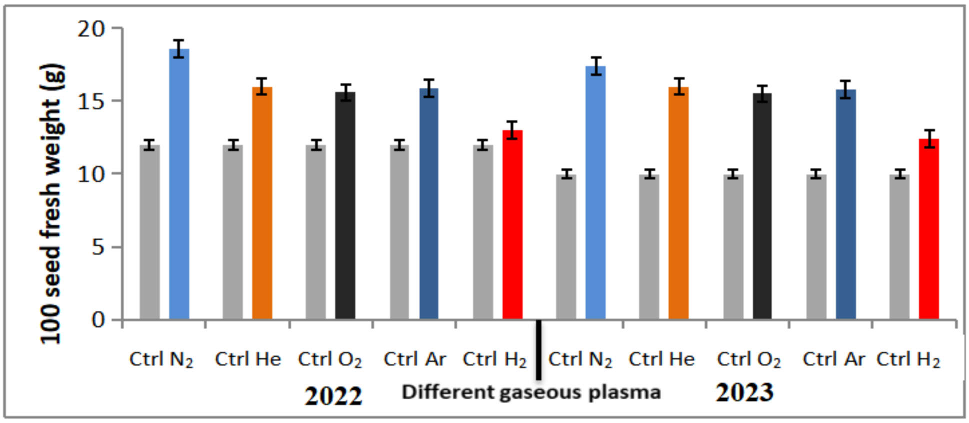


(d)

Fig. 7. Plant fresh weight (a), no. of pods/plant (b), no. of seeds/plant (c) and 100 seeds weight (d) after 90 days of sowing of plasma pre-treated seeds.

(a) (b)

(c)

Fig. 8. After 90 days of sowing of plasma pre-treated seeds: 30 days old plant (a), 60 days old plant (b), 90 days old plant (c) in the field.

PLASMA SOURCES DIAGNOSTICS

SEM

The scanning electron microscope was employed to compare the control seeds with those treated with plasma, aiming to discern alterations in seed surface topology, structure, and overall surface characteristics. Untreated seeds were prepared under identical conditions to serve as the control in the Figure 9, with the exception of plasma treatment. SEM analysis validated the non-invasiveness of plasma treatment, indicating minimal alterations in seed surface morphology across various plasma-treated seeds. Among the sources examined, specific plasma treatments emerged as the most suitable for enhancing soybean seed germination, while also demonstrating efficiency in treatment duration and energy usage.

During non-thermal plasma treatment, plant seeds experience minimal stress, remaining undamaged while being activated by the plasma. This process either enriches the seed surface with oxygen-containing functional groups or causes slight surface etching through ion bombardment [21]. These alterations notably increase surface hydrophilicity, leading to enhanced seed germination, metabolism, and improved permeability for additional supplements [15, 22].

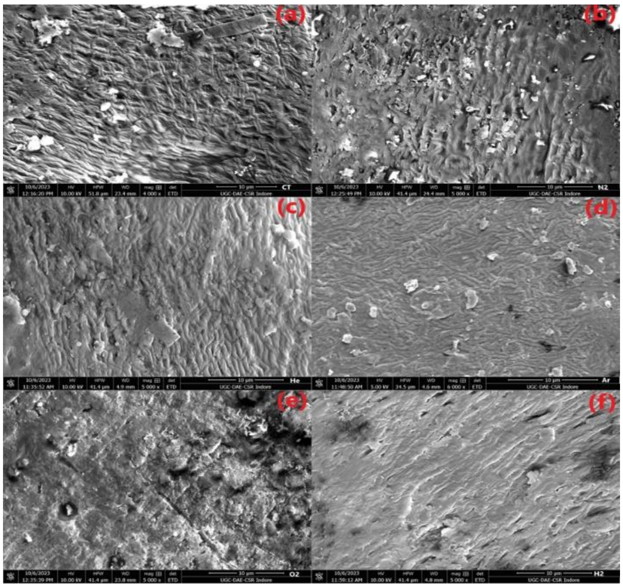
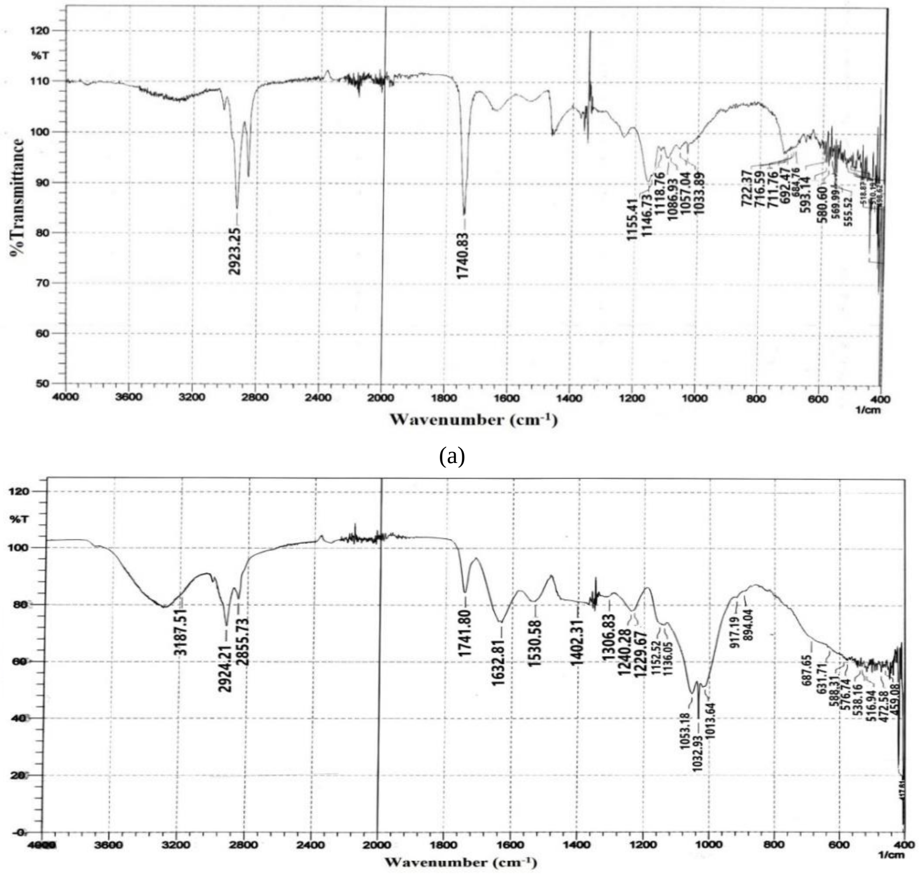


Fig. 9. SEM characterization for control (a), N_2 (b), He (c), Ar (d), O_2 (e), H_2 (f) plasma pre-treated seeds.

FTIR

This study employs FTIR spectroscopy as a rapid and non-destructive analytical tool within the spectrum range of 4000 cm^{-1} to 400 cm^{-1} to investigate the functional groups and polar compounds present in soybean seed samples. Given the complex chemical structure of soybean seeds, the standard IR spectrum effectively delineates its chemical composition, enabling unique identification of the plant. Figure 10 compiles the averaged FTIR spectra of treated soybean seed with various plasma types. The O–H stretching vibrations, which indicate the presence of hydroxyl groups, appear in the peaks between 3200 and 3600 cm⁻¹, related to moisture, alcohols, and phenols [2]. Peaks between 2850 and 2950 cm⁻¹ correspond to C–H stretching vibrations, commonly found in aliphatic hydrocarbons, such as those in lipids (fats and oils). The 1630–1650 cm⁻¹ range reflects C=O stretching vibrations, often seen in carbonyl compounds and proteins (amide I band). Peaks between 1550 and 1570 cm⁻¹ are due to N–H bending vibrations (amide II band), which are typical of proteins [7]. Lastly, the 1030–1240 cm⁻¹ range shows peaks related to C–O and C–C stretching vibrations, indicating the presence of polysaccharides like starch, cellulose, or other carbohydrates [4].



(a)

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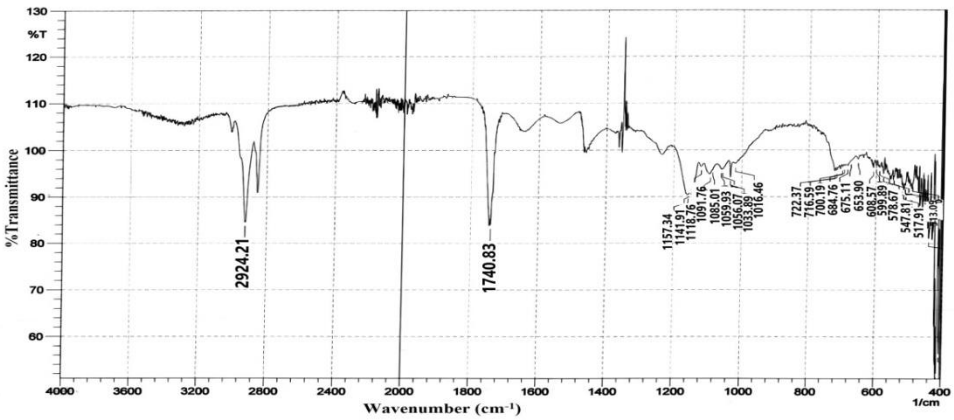
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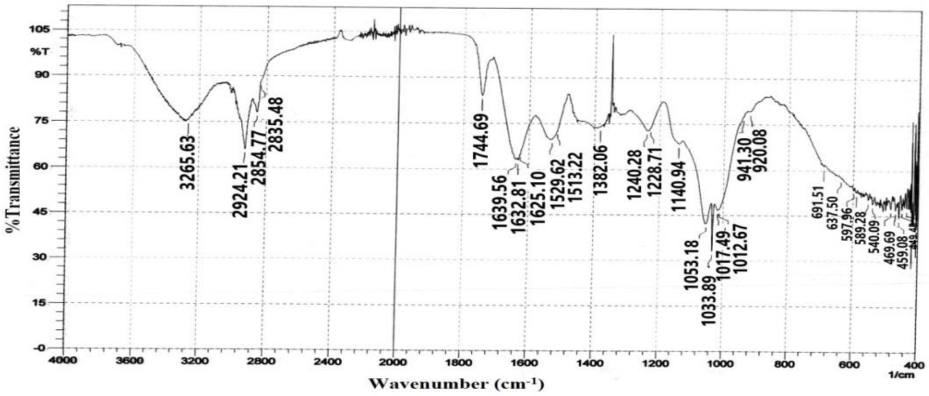
(c)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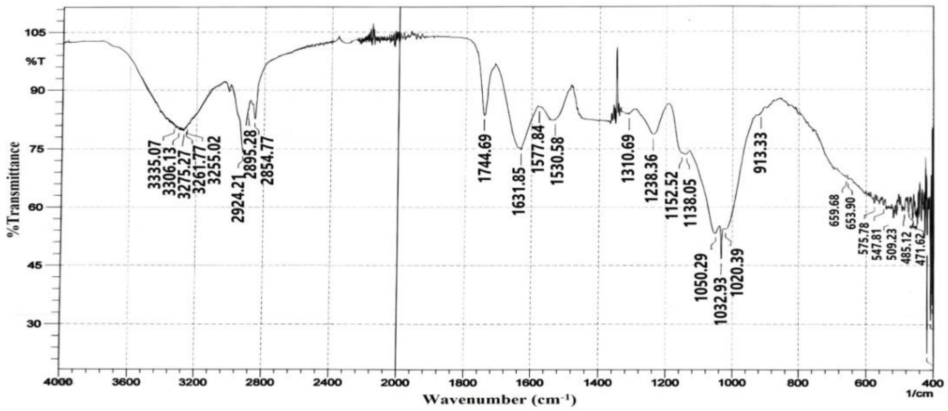
Wavenumber (cm⁻¹)

(d)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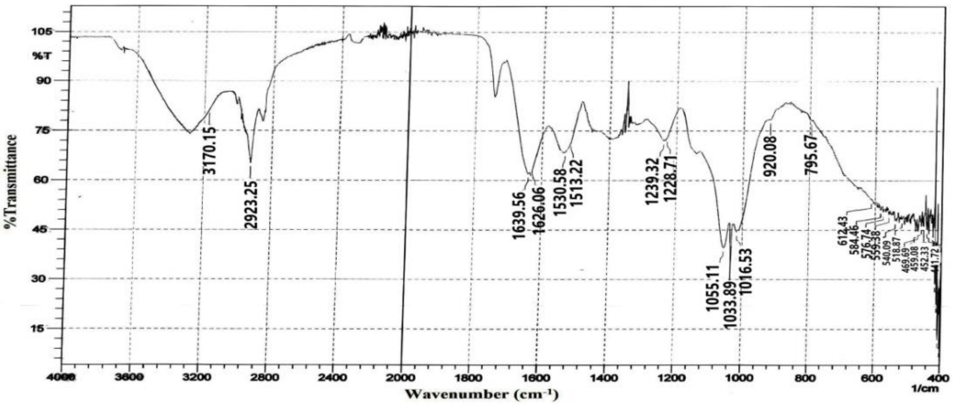
3335.07
3306.13
3275.27
3261.77
3255.02
2924.21
2895.28
2854.77
1744.69
1631.85
1577.84
1530.58
1310.69
1238.36
1152.52
1138.05
1050.29
1032.93
1020.39
913.33
659.68
653.90
575.78
547.81
509.23
485.12
471.62
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm
Wavenumber (cm⁻¹)

(e)



(f)

Fig. 10. FTIR of control (a), nitrogen (b), helium (c), argon (d), oxygen (e), and hydrogen (f) plasma pre-treated seeds.

Variation among soybean seed samples is discerned through spectral data analysis. The graph illustrates six distinct samples of soybean seed powder's infrared spectrogram, the spectra are presented in the 4000 cm^{-1} to 400 cm^{-1} with precise averaged peak locations indicating distinctive fingerprint characteristics, particularly between 1300 cm^{-1} and 1700 cm^{-1}, and 1000 cm^{-1} and 1200 cm^{-1}. Notably, wide bands appear at wavelengths between 3300 cm^{-1} and 3000 cm^{-1}, mid-strong bands between 3000 cm^{-1} and 2800 cm^{-1}, an intense band at 1745 cm^{-1}, and several overlapping bands between 1800 cm^{-1} and 600 cm^{-1}. Comparison of the infrared spectra of six soybean seed samples treated with different plasma types reveals discrepancies in absorption band locations or intensities despite variations in sample forms. Detailed analysis shows distinctive features:

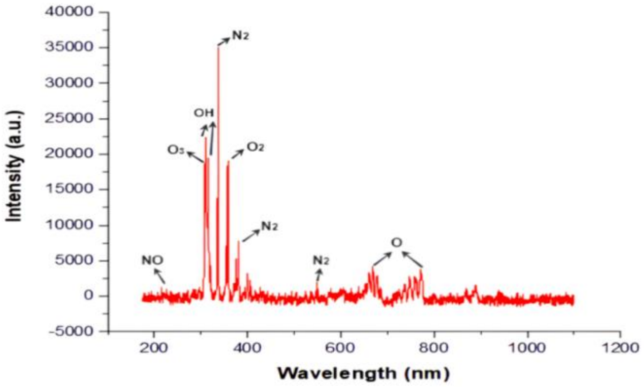
Control samples exhibit a prominent peak at 2923 cm^{-1}, corresponding to the C–H stretching vibration due to presence of fatty acids or lipids. A peak at 1740 cm^{-1} is characteristic of C=O stretching vibration related to triglycerides. Similarly, Nitrogen plasma-treated samples show peaks at 2924 cm^{-1} and 1740 cm^{-1}. The helium plasma-treated samples display signal at 3187 cm^{-1}, which corresponds to moisture content like water, alcohols, or carboxylic acids with O–H stretching vibration, and other signals at 1741 cm^{-1}, 1632 cm^{-1}, 1530 cm^{-1}, and 1402 cm^{-1} which are the presence of triglycerides, protein amide-I, protein amide-II, and fatty acids corresponding to C=O stretching and N–H bending vibration. The peak at 1402 cm^{-1} is related to the bending vibrations in aliphatic chains or symmetric stretching in carboxylate group. Oxygen, argon, and hydrogen plasma-treated samples exhibit almost identical peaks from 3255 to 3335 cm^{-1} associated with O–H stretching vibration, 2850–2950 cm^{-1} shows the stretching vibrations of C–H,1744 cm^{-1} C=O stretching, 1630–1650 cm^{-1} peak represents C=O stretching vibrations, 1550–1570 cm^{-1} suggest the N–H bending vibrations. In some samples the peaks at

1016 cm^{-1} and 1055 cm^{-1} are showing the characteristic of the sugar backbone vibrations (C–C or C–O stretching) within polysaccharides or cellulose components. Various other peaks reflect the presence of different chemical groups and compounds, such as carbonyl chemicals, aromatic rings, aliphatic C–H stretching groups, polysaccharides, coumarin, alcohols, and benzene rings.

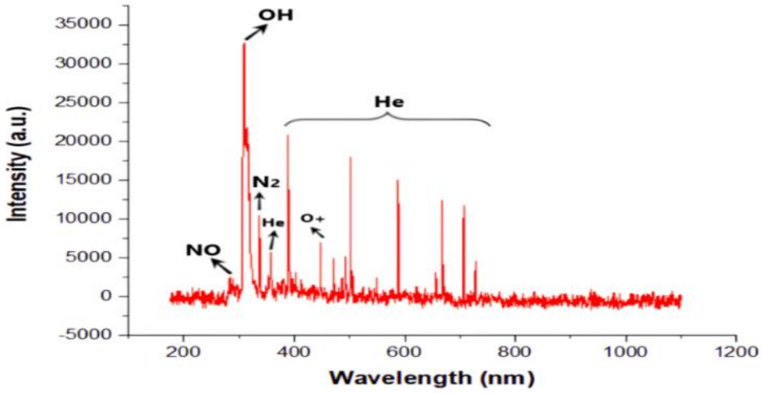
The IR spectra provide valuable insights into the chemical composition of soybean seeds, with the samples that were treated with plasma showing a greater concentration of proteins, carbohydrates, and possibly aromatic compounds than the control sample. Plasma treatment disrupts the lipids.

OES

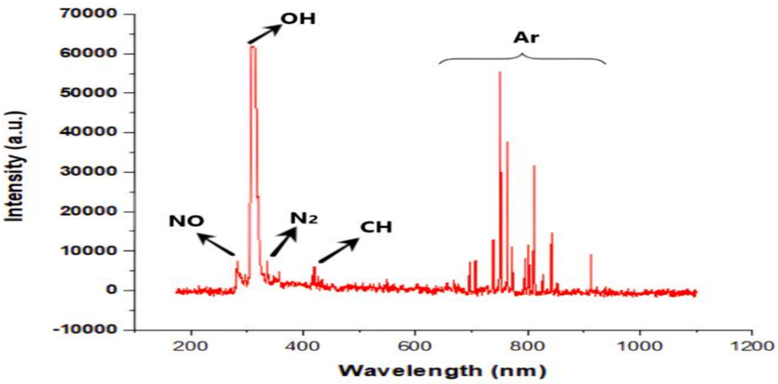
Figure 11 employs optical emission spectroscopy (OES) to illustrate the reactive oxygen and nitrogen species (RONS) generated during plasma in the gaseous phase with various gas types. During O_2 plasma, a robust OH peak at 306.9 nm with high intensity, a minor NO peak at 200–280 nm, and the singlet oxygen (O) positive system at 777.2 nm and 844.1 nm were observed. In N_2 plasma, OH peaks at 306.9 nm and 309 nm, prominent peaks at 358 nm, and N_2 peaks in the range of 500–700 nm were detected. Additionally, Ar plasma exhibited a significant OH peak at 309.6 nm and additional Ar lines within the range of 650–850 nm. Air plasma displayed small emission lines in the range of 200–250 nm, attributed to the molecular NO β, γ system, along with strong N_2 peaks at 350–380 nm and CH peaks at 420 nm. In helium plasma, a robust OH peak at 309 nm with high intensity, a major NO peak at 280 to 290 nm, and He peaks at 388 nm, 502 nm, 588 nm, 667 nm, and 706 nm were observed. For hydrogen plasma, the largest OH peak was at 309 nm, with small NO peaks spanning 200–300 nm, and the presence of H_α and H_β at 656 nm and 486 nm, respectively, along with H_2 peaks between 580 to 650 nm. These reactive species, particularly oxygen and nitrogen, significantly facilitated the sterilization process.



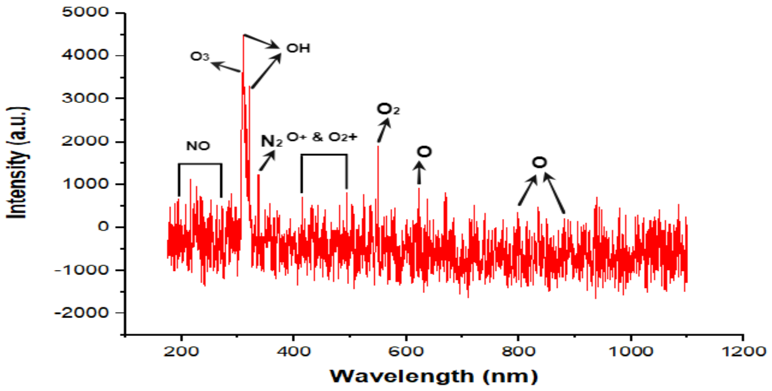
(a)


Intensity (a.u.)
35000
30000
25000
20000
15000
10000
5000
0
-5000
OH
He
N₂
He
O+
NO
200
400
600
800
1000
1200
Wavelength (nm)

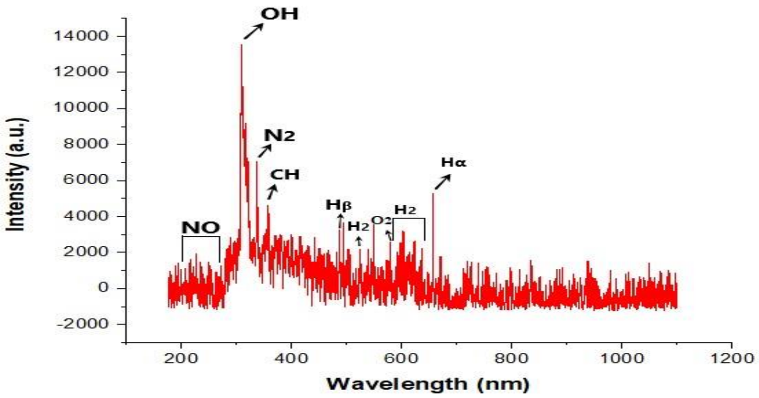
(b)


Intensity (a.u.)
70000
60000
50000
40000
30000
20000
10000
0
-10000
OH
Ar
NO
N₂
CH
200
400
600
800
1000
1200
Wavelength (nm)

(c)


Intensity (a.u.)
5000
4000
3000
2000
1000
0
-1000
-2000
O₃
OH
NO
N₂
O+ & O₂+
O₂
O
O
200
400
600
800
1000
1200
Wavelength (nm)

(d)



(e)

Fig. 11. OES of nitrogen (a), helium (b), argon (c), oxygen (d), and hydrogen (e) plasma pre-treated seeds.

DISCUSSION

Pre-sowing treatment of seeds with plasma was shown to be effective in promoting productivity in crop plants like tomatoes [19], corn [1], and legumes [3]. The results presented here illustrate the effectiveness of O_2, N_2, He, Ar, and H_2 plasma on soybean seeds. A detailed comparative analysis of all the gases used here had not been under taken earlier at the field level on the growth and yield of soybean. It is evident from the data that plasma. Pre-sowing treatment of seeds enhances the growth and yield of soybean irrespective of the type of the gas used to produce the plasma. The differences are quantitative between the gases. In terms of yield, N_2 plasma proved to be the most effective by promoting the production to over 60 % in the number of seeds obtained per plant and the weight of 100 seeds (Fig. 7, Table 3). H_2 plasma proved to be the least effective promoting the number of seeds per plant by 17 % and seed weight by 15 % (Fig. 7, Table 3). The other three gases (He, O_2, and Ar) produced an effect that falls in between these two extremes; between 35 to 45 % promotion approximately. It is evident that except for H_2 plasma, all the gases here have a significant promotive effect on the growth and yield of soybean at the field level. The results obtained have been consistent for two years of Kharif season and pre-treatment with plasma can be recommended as a safe environmentally friendly method for enhancing the yield of soybean.

SEM analysis validated the non-invasiveness of plasma treatment, indicating minimal alterations in seed surface morphology across various plasma-treated seeds. Among the sources examined, specific plasma treatments emerged as the most suitable for enhancing soybean seed germination, while also demonstrating efficiency in treatment duration and energy usage. FTIR spectroscopy indicated modifications after exposure to plasma-treated seeds, suggesting surface activation due to lipid breakdown [26]. OES detected OH, NO, O, and N_2 radicals during plasma treatment, contributing to the optimal outcome.

CONCLUSION

The experiments with pre-treatment of soybean seeds (Glycine max var. JS-9560) have successfully demonstrated the promotive effect of N_2, He, O_2, Ar, and H_2 on the growth and yield at the field level consistently for two years (2022 and 2023). Except for H_2 plasma, the other four gases have the ability to enhance the yield by over 40 % to 60 % depending on the type of gas used to produce plasma. Plasma pre-treatment is a safe and ecologically compatible method to improve the yield of soybean.

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REFERENCES

1. AHN, C., J.J.B. GILL, D.N. RUZIC, Growth of plasma-treated corn seeds under realistic conditions, *Scientific Reports*, 2019, **9**(1), https://doi.org/10.1038/s41598-019-40700-9.
2. ANDRADE, G.C., C.M.M. COELHO, V.G. UARROTA, Modelling the vigour of maize seeds submitted to artificial accelerated ageing based on ATR-FTIR data and chemometric tools (PCA, HCA and PLS-DA), *Heliyon*, 2020, **6**(2), e03477, https://doi.org/10.1016/j.heliyon.2020.e03477.
3. CHEN, H.H., Y.K. CHEN, H.C. CHANG, Evaluation of physicochemical properties of plasma treated brown rice, *Food Chemistry*, 2012, **135**(1), 74–79, https://doi.org/10.1016/j.foodchem.2012.04.092.
4. CUETO, M., A. FARRONI, S.D. RODRÍGUEZ, R. SCHOENLECHNER, G. SCHLEINING, M. DEL PILAR BUERA, Assessing changes in enriched maize flour formulations after extrusion by means of FTIR, XRD, and chemometric analysis, *Food and Bioprocess Technology*, **11**(8), 1586–1595, https://doi.org/10.1007/s11947-018-2113-6.
5. DHAYAL, M., S.Y. LEE, S.U. PARK, Using low-pressure plasma for Carthamus tinctorium L. seed surface modification, *Vacuum*, 2006, **80**(5), 499–506, https://doi.org/10.1016/j.vacuum.2005.06.008.

6. FILATOVA, I.I., V.V. AZHARONOK, M.A. KADYROV, V. BELJAVSKY, A. GVOZDOV, A. SHIK, A.E. ANTONUK, N. BELARUS, The effect of plasma treatment of seeds of some grain and legumes on their sowing quality and productivity, *Rom. Rep. Phys.*, 2011, **56**, 139–143.

7. GHASEMI, M., M.A. MIRI, M.A. NAJAFI, M. TAVAKOLI, T. HADADI, Encapsulation of cumin essential oil in zein electrospun fibers: Characterization and antibacterial effect, *Journal of Food Measurement & Characterization*, 2022, **16**(2), 1613–1624, https://doi.org/10.1007/s11694-021-01268-z.

8. GÓMEZ-RAMÍREZ, A., C. LÓPEZ-SANTOS, M. CANTOS, J.L. GARCÍA, R. MOLINA, J. COTRINO, J. ESPINÓS, A.R. GONZÁLEZ-ELIPE, Surface chemistry and germination improvement of quinoa seeds subjected to plasma activation, *Scientific Reports*, 2017, **7**(1), https://doi.org/10.1038/s41598-017-06164-5.

9. JIANG J., X. HE , L. LI , J. LI, H. SHAO, Q. XU, R. YE, Y. DOG, Effect of cold plasma treatment on seed germination and growth of wheat, *Plasma Science and Technology*, 2014, **16**(1), 54-58, https://doi.org/10.1088/1009-0630/16/1/12.

10. JIANG, J., Y. LU, J. LI, L. LI, X. HE, H. SHAO, Y. DONG, Effect of seed treatment by cold plasma on the resistance of tomato to *Ralstonia solanacearum* (Bacterial Wilt), *PLoS ONE*, 2014, **9**(5), e97753, https://doi.org/10.1371/journal.pone.0097753.

11. LING, L., J. JIAFENG, L. JIANGANG, S. MINCHONG, H. XIN, S. HANLIANG, D. YUANHUA, Effects of cold plasma treatment on seed germination and seedling growth of soybean, *Scientific Reports*, 2014, **4**(1), https://doi.org/10.1038/srep05859.

12. LIU, B., B. HONNORAT, H. YANG, J.A. MONREAL, L. RAJJOU, A. ROUSSEAU, Non-thermal DBD plasma array on seed germination of different plant species, *Journal of Physics D Applied Physics*, 2018, **52**(2), 025401. https://doi.org/10.1088/1361-6463/aae771.

13. MEIQIANG, Y., M. HUANG, M. BUZHOU, M. TENGCAI, Stimulating effects of seed treatment by magnetized plasma on tomato growth and yield, *Plasma Science & Technology*, 2005, **7**(6), 3143–3147, https://doi.org/10.1088/1009-0630/7/6/017.

14. OTHMAN, K.B., M.M. CHERIF, I. ASSADI, W. ELFALLEH, L. KHEZAMI, A. GHORBAL, A.A. ASSADI, Exploring cold plasma technology: Enhancements in carob seed germination, phytochemical composition, and antioxidant activity, *Heliyon*, 2024, **10**(8), e28966, https://doi.org/10.1016/j.heliyon.2024.e28966.

15. PAVLOVICH M. J., T. ONO, C. GALLEHER, B. CURTIS, D.S. CLARK, Z. MACHALA, D.B. GRAVES, Air spark-like plasma source for antimicrobial NOx generation, *Journal of Physics D Applied Physics*, 2014, **47**(50), 505202, https://doi.org/10.1088/0022-3727/47/50/505202.

16. RADHAKRISHNAN, R., B.D. RANJITHA KUMARI, Pulsed magnetic field: A contemporary approach offers to enhance plant growth and yield of soybean, *Plant Physiology and Biochemistry*, 2012, **51**, 139–144, https://doi.org/10.1016/j.plaphy.2011.10.017.

17. REUTER, S., J.S. SOUSA, G.D. STANCU, J.P. HUBERTUS, Review on vuv to mir absorption spectroscopy of atmospheric pressure plasma jets, *Plasma Sources Science and Technology*, 2015, **24**(5),054001, https:// doi.org/10.1088/0963-0252/24/5/054001.

18. SERA, B., P. SPATENKA, M. SERY, N. VRCHOTOVA, I. HRUSKOVA, Influence of plasma treatment on wheat and oat germination and early growth, *IEEE Transactions on Plasma Science*, 2010, **38**(10), 2963–2968. https://doi.org/10.1109/tps.2010.2060728.

19. SERÁ, B., V. STRANÁK, M. SERÝ, M. TICHÝ, P. SPATENKA, Germination of Chenopodium album in response to microwave plasma treatment, *Plasma Science and Technology*, 2008, **10**(4), 506–511, https://doi.org/10.1088/1009-0630/10/4/22.

20. SHINE, M., K.N. GURUPRASAD, A. ANAND, Enhancement of germination, growth, and photosynthesis in soybean by pre-treatment of seeds with magnetic field, *Bioelectromagnetics*, 2011, **32**(6), 474–484, https://doi.org/10.1002/bem.20656.

21. TONG, J., R. HE, X. ZHANG, R. ZHAN, W. CHEN, S. YANG, Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of *Andrographis paniculata*, *Plasma Science and Technology*, 2014, **16**(3), 260–266, https://doi.org/10.1088/1009-0630/16/3/16.

22. VARNAGIRIS, A., S. VILIMAITE, I. MIKELIONYTE, M. URBONAVIČIUS, S. TUČKUTĖ, D. MILČIUS, The combination of simultaneous plasma treatment with Mg nanoparticles deposition technique for better mung bean seeds germination, *Processes*, 2020, **8**(12), 1575, https://doi.org/10.3390/pr8121575.

23. ZHOU, Z.W., Y.F. HUANG, S.Z. YANG, D.Y. XIONG, *Progress in Electromagnetics Research Symposium Proceedings*, Kuala Lumpur, Malaysia, 2012, **1577**, ISBN: 978-1-934142-19-6.

24. ZHOU, Z., Y. HUANG, G. XU, K. BO, Effects of plasma treatment of maize seeds (No. 2-Zhunuo) on the biological properties and yield, *Agricultural Sciences*, 2020, **11**(04), 431–439, https://doi.org/10.4236/as.2020.114026.

25. ZHOU, Z., Y. HUANG, S. YANG, W. CHEN, Introduction of a new atmospheric pressure plasma device and application on tomato seeds, *Agricultural Sciences*, 2011, **02**(01), 23–27, https://doi.org/10.4236/as.2011.21004.

26. ZHU, X., P.U. YI-KANG, A simple collisional-radiative model for low-temperature argon discharges with pressure ranging from 1 Pa to atmospheric pressure: Kinetics of Paschen 1s and 2p levels, *Journal of Physics D Applied Physics*, 2010, **43**(1), 015204, DOI: 10.1088/0022-3727/43/1/015204.