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Research article

Impact of LFGD (Ar+O_2) plasma on seed surface, germination, plant growth, productivity and nutritional composition of maize (*Zea mays* L.)

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ABSTRACT

In this present study conducted with the LFGD (Low-Frequency Glow Discharge) (Ar + O_2) plasma treated maize seeds, to inspect the effect on seed surface modifications, seed germination, growth, development, productivity and nutritional compositions of maize plants. This study reported that LFGD (Ar + O_2) plasma treated maize seeds have a potential effect to change its smooth seed surfaces and, it becomes rougher. It also enhances the seed germination rate up to (15.88%), which might help to increase the shoot length (33.42%), root length (10.67%), stem diameter (13.37%), total chlorophyll content (46.93%), total soluble protein (52.48%), total soluble phenol (21.68%) and sugar (1.62%) concentrations in respect controls of our experimental plants. For this reason, the acceptable treatment duration for maize seeds were 30sec, 60sec, 90sec and 120sec. After treatment, the plants exhibited a significant increase in CAT, SOD, APX and GR activities in the leaves and roots, and also significantly changes in H_2O_2 (208.33 ± 5.87μ $molg^{-1}$ FW) in the leaves and (61.13 ± 1.72μ $molg^{-1}$ FW) in the roots, NO was (369.24 ± 213.19μ $molg^{-1}$FW) and (1094.23 ± 135.44μ $molg^{-1}$FW) in the leaves and roots. LFGD plasma treatment also contributed to enhancement of productivity (1.27%), nutritional (moisture, ash, fat, and crude fiber) compositions, and iron and zinc micro-nutrition concentrations of maize. From this research, LFGD (Ar + O_2) plasma treatment showed a potential impact on the maize cultivation system, which is very effective tools and both in nationally and internationally alter the conventional cultivation system of maize. Because it promotes seed surface modification, improved germination rate, shoot length, root length, chlorophyll content, some of the growths related enzymatic activity, nutrient composition, iron, and zinc micro-nutrients and the productivity of maize.

1. Introduction

Maize (*Zea mays* L.) is one of an extreme popular cereal crop belonging to the family of *Poaceae*. It is extensively cultivated throughout the world not only for its high production but also for its high nutrition value. Maize is an essential nourishment for about 75% of the country's population, and most of the farmers grow maize as an individual crop in their fields [1]. Maize is consumed in roasted and fried form and also used in poultry feed and used as fodder for livestock. Besides, it was used for ethanol, corn starch and corn syrup production in many other countries. The position of maize is 1st among the cereals in terms of its yield [2] but in terms of area and production, it is ranked 3rd just after rice and wheat [3].

Bangladesh is an agriculture-based country in the world. It is a good source of nutrients for the under-nourished and malnourished population in Bangladesh. Maize introduced itself as a poultry feed and fodder for livestock in Bangladesh. The husk from the maize contains cellulose in rich constituents, and it could be possible to produce a biopolymer from maize husk [4].

Due to the decrease of farming land and environmental threats, global crop production is at risk. Production is decreasing day by day due to several climatic and man-made factors [5]. Furthermore, the application

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of undiscriminating chemical nourishments and pesticides, global warming conveys deathtraps to anthropological well-being and the atmosphere. Therefore, environmentally safer policy and expertise should be used in agriculture.

Plasma is one of the four basic stages of the elements. Plasma technology has been shown to guide agronomic improvement in crops. Atmospheric pressure cold plasma has been reported for the improvement of seed germination rate and growth of limited plants species [5]. This technology represents much attention because it excites the biological mechanisms in plants. Plasma treatment have the ability to prompt the seed germination rate, decreases surface infection, and impedes the growth of unwanted pathogens. Seed germination rate is a deciding factor for overall crop productivity which is influenced by imbibition of water uptake leading to break down the dormant stage of the embryo. Numerous physiological, and biochemical vagaries such as synthesis of protein, enzymatic stimulation, and starch digestions are involving in seed germination activity [6]. Treatment of seeds with specific plasma, triggers specific biochemical changes and also induce positive physical changes on seed ultimately enhances germination rate.

It has been reported that, water uptake mechanisms for the enhancement of seed germination atmospheric pressure cold plasmas (APCPs) were very useful tools [7, 8]. The APDBD plasma and the LPDBD technique are two most widely used non-thermal plasma. Cold plasma is also a promising tool in medical science, among them argon based plasma jet is used for cancer treatment both *in vivo* and *in vitro* [9, 10].

Earlier reports show that cold plasma has the positive effect on the germination and growth of soybean seeds [11, 12, 13] oat seeds [12], oilseed rape [14], beans, lentils [15, 16], hemp [17] and other agronomical and economically important crops. LFGD (Low-Frequency Glow Discharge) plasma is also a type of non-thermal plasma and gaining increasing interest in crop improvement. It was also reported that LFGD plasma enhances the seed germination rate and growth of wheat [17]. However, the mechanism of LFGD plasma was not yet examined in any other plant species for seed germination, growth and development, and the enzymatic activity. Besides, it is always interesting to increase the nutritional content such as; moisture, ash, fat and fiber, and also increased zinc and iron micro-nutrients of the grain in F1 generation for sustainability.

The objective of the current study is to increase the germination rate, growth and development of maize and also improve its nutrient composition using LFGD plasma.

2. Materials and methods

2.1. Plasma production

In Figure 1, displayed the schematic for the plasma generation reactor, which was made of a glass pipe specially Pyrex glass. The length of this reactor is approximately 9cm and the diameter is about 4.5 cm. One end of the pipe was sealed with a stainless steel (SS) circular disk and used as a power electrode, while the other end was covered with another SS circular disk that can be opened for filling up the reactor with seeds and used as grounded electrode. A servo-motor was used to rotate the pipe horizontally in order to treat the seed surface uniformly. The reactor was then placed inside a bell jar and a rotatory propel was used to vacate the bell jar. The pressure was 400 torr inside the bell jar was maintained for the intact seed treatment durations.

A sinusoidal bipolar power supply (0–10kV, 8 kHz) was used for the production of glow discharge (Ar + O_2) plasma, as shown in Figure 1 inside the seed treatment reactor. Maize seeds were inserted in discharge pipe in the middle of the two electrodes. In Figure 1, the entire circumstance of the reactor was employed in a vacuum compartment. The exclusive force of the compartment was abridged by a vacuum pump (FY-1C) and maintained ~400 torr. The flow of Ar and O_2 gases in the compartment were measured by two gas flow meters (Yamato was used for Ar gas and KIT115P was used for O_2).

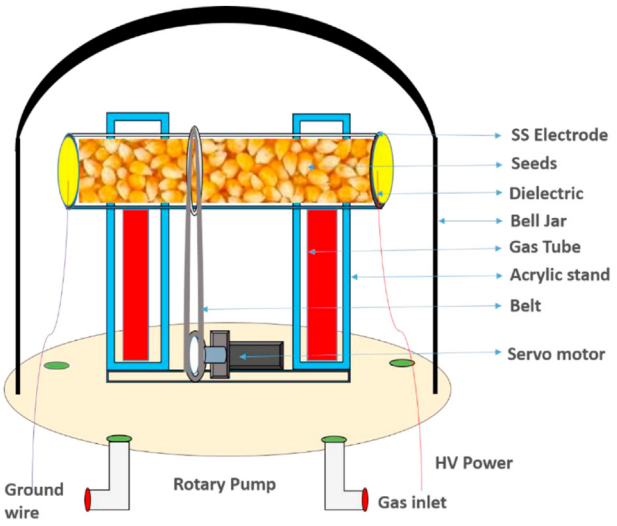

Figure 1. Schematic diagram of the setup of 400 torr glow discharge (Ar + O_2) plasma seed treatment reactor in laboratory condition.

2.2. Specification of generated plasma

Inside the seed treatment reactor, the emission spectrum of Ar and O_2 discharge at 400 torr is presented in Figure 2. Voltage and current were measured with a digital oscilloscope (RIGOL: DS 1104, Japan) in combination with the respective probes. The utilized power for the production of plasmas was measured by

$$P = \int_0^T v(t)i(t)dt, \tag{1}$$

where $v(t)$ and $i(t)$ are the voltage and current, respectively. Power used in discharge was $\sim 45W$ for the electrode spacing of 60 mm at a discharge voltage of $5kV$ and frequency of $5kHz$. The emitted spectra produced in the plasma was recorded with spectrometers (Ocean Optics: USB2000 + XR1, slit size: $25\mu m$, grating: 800 *lines/mm*, optical resolution: 1.7 *nm*, wavelength range: 200–1100nm and high resolution dual-channel spectrometer AvaSpec-2018, slit: $10\mu m$, grating: 2400 lines/

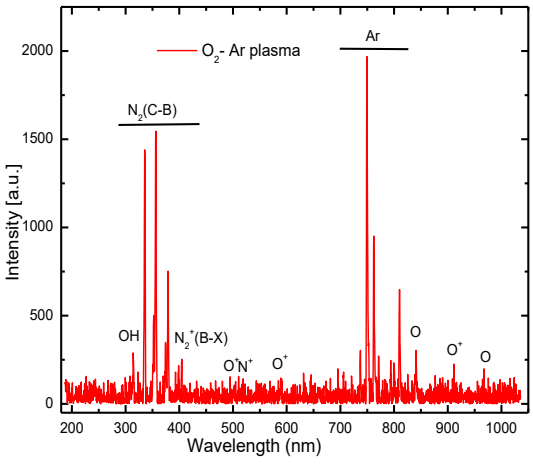

Figure 2. Emission spectra of LFGD (Ar + O_2) plasma dignified at 5 kV, 400 torr and the space between electrodes were 60mm.

mm, optical resolution: 0.07nm, wavelength range: 220–800nm) for plasma specification and plasma diagnostics.

2.3. Land preparation

The field experiment was conducted during the 2018 late cropping season from December to March at The Research Field of Rajshahi University. Three ploughings were used for clearing land, weeding and surface drains were provided before seeds sowing. The area of each plot was $4m^2$ (2m X 2m). There were three replications in each treatment which were planted randomly in the field.

2.4. Collection of seeds and plasma treatment

Maize seeds MIRAKKLE-300 were collected from The Bangladesh Agricultural Development Council, Rajshahi. It is a hybrid variety, the germination rate of this variety is approximately 78%, the grain color is golden yellow, the plant height range 95–120cm, duration 105–135 days, weight of 1000 grains 200–240g. The selected maize seeds were positioned in a tube-shaped glass vessel finished of a 12mm innermost width glass container. The magnitude of the glass vessel was 50mm whereas the superior adjacent was open, and the inferior adjacent remained enclosed with a lattice ended of cotton. The exploration of seeds was completed in such a way that when the container rotated like a rotating top, each of the seeds got the opportunity for optimal surface treatment. The maize seeds were treated for 30s, 60s, 90s, and 120s respectively.

2.5. SEM (scanning electron microscope) analysis from LFGD plasma treated seeds

The maize seeds were treated with LFGD plasma at 30s, 60s, 90s and 120s. A high-resolution FEI S50 microscope scanning through the passes of electron (EVO-18, Carl Zeiss, Germany) used to investigate the seed surface structure. The images were taken using 10 kV accelerating voltage with 2.50 K × magnification.

2.6. Seed germination rate

In case of plasma treatment, these two gasses' (argon and oxygen) compounds can easily interact with the surface of the maize seeds and enhance the surface properties [17]. It also increased the surface hydrophilicity of the seeds [18]. After LFGD ($Ar + O_2$) plasma treatment, the seeds (around 40) were sown to the plot for germination. Irrigation was given to the field to conserve adequate wetness for maize seeds germination. The maize seed germination rate was calculated by the following Eq. (2).

$$\text{Germination rate (\%)} = (\text{Number of seeds germinated} / \text{Total number of seeds}) \times 100\ \% \quad (2)$$

2.7. Morphological features and the determination of chlorophyll content

The morphological feature like; shoots and roots length were perceived using a centimeter ruler after 15, 30 and 45 days after swing to the field. Morphological features of the plant such as the number of leaves and stem diameter were also recorded in a regular interval (15, 30, 45 days of swing). The roots and shoots were reserved in an oven for 72 h at 70 °C to govern the dry weightiness. After 7 days, shoots and roots dry weightiness were dignified using HT analytical balance. The approximation of chlorophyll concentration, young fresh leaves were grounded (mix with 90% methanol) using a mortar and pestle. Then the mixture was centrifuged at 12000 rpm for 5 min, and collected the supernatant in a fresh tube. The absorbance was taken at 662nm (chlorophyll *a*) and 646nm (chlorophyll *b*) using a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, USA). The total concentrations of chlorophyll *a* and chlorophyll *b* was calculated following the method described by Lichtenthaler and Wellburn [19].

2.8. Hydrogen peroxide (H_2O_2) determination

After measuring the weight of the leaf, added 0.1 % trichloroacetic acid (TCA) for homogenization used a mortar and pestle [20], and centrifuging at 10000 rpm for 15 min. Potassium iodide (1M) and phosphate buffer (10mM, pH 7.0) were used to mix the collected supernatant and kept the mixture in a dark place for 1 h. Finally, the absorbance was taken at 390nm in a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific, MA, USA).

2.9. Nitric oxide (NO) analysis

The NO was analysed raised on the modification from oxyhemoglobin (HbO_2) to methemoglobin (metHb) for ensuring the presence of NO [21]. The collected shoots and roots were stranded in 1ml of refrigerated NO buffer, which comprises 0.1 M sodium acetate, 1 M NaCl, and 1% (w/v) ascorbic acid (pH 6.0). The mixture was centrifuged in a cooling centrifuge at 10,000 rpm for 5 min at 4 °C. Afterward, the samples were mixed with the HbO_2 solution (5mM) and incubated at room temperature for 5min. Finally, the absorbance was taken at 401nm in a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific, MA, USA).

2.10. Estimation of total soluble sugar

The total soluble sugar in leaves and roots were investigated as earlier described [22]. The leaves and roots sample were homogenized in 80% ethanol and then centrifuged at 12000 rpm for 5 min. Then added 0.2% of anthrone component, the mixture incubated in a steam bath used for 8min and then positioned on ice. Finally, the absorbance was taken at 620nm in a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific, MA, USA).

2.11. Estimation of total soluble protein

The total soluble protein in the leaves and roots were investigated as earlier described [23]. In brief, weighed the leaves and roots were grounded in ice-cold mortar and pestle. Then added 50 mMTris-HCl, 2 mM EDTA (ethylene di-amine tetra acetic acid) buffer and 0.04% (v/v) 2-mercaptoethanol, where pH was maintained 7.5. Then the mixture was centrifuged at 12000 rpm for 10 min at 25 °C. The 100 µl supernatant was poured to a quartz cuvette containing 1 ml CBB (Coomassie Brilliant Blue). Finally, the absorbance was taken at 595nm in a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific, MA, USA).

2.12. Estimation of total soluble phenol

The total soluble phenol in the leaves and roots were investigated as earlier described [24]. Approximately, 50 mg homogenized leaves and roots samples were centrifuged at 12000 rpm for 10 min. In this assay, 250 µl of Folin-Ciocalteus phenol reagent was added to 50 ul of root and leaf sample homogenate supernatant. Subsequently, 20% of aquous solution of Na_2Ca_3 (14.3/500ml) was added to this mixture and gently vortexed. Finally, 500µl of water was added and incubated at 25 °C for 30 min. The absorbance was taken at 765 nm in a spectrophotometer (Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific, MA, USA).

2.13. Analysis of antioxidant enzymes (CAT, SOD and APX) from leaf and root of the plants

CAT, SOD and APX enzymes in the leaves and roots were investigated as earlier described [25]. Briefly, the leaves and roots sample were ground added phosphate buffer (100 mM, pH 7.0) using a mortar and pestle. Then centrifuged at 12000 rpm for 10 min. The CAT was investigated in a reaction mixture contained 100 μl of leaves and roots extract, 100mM potassium phosphate buffer (pH 7.0) and 6% H_2O_2. The absorbance was taken at 240nm in a spectrophotometer (Genesys 10S, Thermo Scientific, MA, USA) at 30 s intervals up to 1 min where the standard coefficient of the extinction was maintained 0.036 mM^{-1} cm^{-1}. For the estimation of SOD, 100μl leaves and roots extract mixed with 0.1 mM EDTA, 50mM sodium carbonate buffer (pH 9.8), and 0.6 mM epinephrine [26]. The absorbance was taken at 475nm after 4 min intervals using a spectrophotometer (Genesys 10S, Thermo Scientific, MA, USA). The APX was estimatesd as earlier described [27]. The mixture having 0.1 ml of leaves and roots extract, 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbic acid, 0.1 mM H_2O_2. The absorbance was taken at 290nm in UV-VIS spectrophotometer (Genesys 10S, Thermo Scientific, MA, USA) at 30 s intervals. Where, the coefficient of the extinction was maintained 2.8 mM^{-1} cm^{-1}. The GR was estimatesd as earlier described [27]. The mixture having 100 μl of leaves and roots extract, 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.75 ml distilled water, 0.1 ml of 20 mM oxidized glutathione (NADPH) and 0.1 ml of 2mM NADPH. The absorbance was taken at 340nm in UV-VIS spectrophotometer (Genesys 10S, Thermo Scientific, MA, USA) at 30 s intervals.

2.14. Yield of the maize

The average number of grains and the average weight of grains per cob were counted manually and weighted by HT analytical balance (Vibra, Shinko Denshi Company Limited, Japan).

2.15. Determination of nutritional compositions (moisture, ash, fat and crude fiber) contents

2.15.1. Moisture content

At first, the empty dish and lid was heated at 105 °C for 3 h in a 300 °*C incubator* and transferred to desiccators to cool. Three grams of the sample was spread to the uniformity of the dish and placed in the oven. The samples were dried at 105 °C for 3 h. Then it was transferred to a moderately protected desiccator for cooling. Dried samples were reweighted. Finally, the moisture content was calculated by the following Eq. (3).

$$\text{Moisture (\%)} = (W1–W2/W1) *100 \quad (3)$$

2.15.2. Ash content

The lid and crucible were heated at 550 °C overnight to confirm that layer on the exterior of the crucible was blackened off. The lid and crucible were subjected to 3-unit places. 5 g of the sample was taken in the crucible and heated over low Bunsen flam half covered with the lid. Finally, the desiccator was cooled down and the ash with lid and crucible were weighted. When the sample turned to gray, the lid and crucible were reverted to the oven for further ashing. The ash content was calculated by the following Eq. (4).

$$\text{Ash (\%)} = (\text{Weight of ash/Weight of the total sample}) *100 \quad (4)$$

2.15.3. Fat content

A 500 ml bottle and cap were excited in the incubator at 105 °C instantaneous to confirm the weight of the bottle is stable. The five-gram of the sample was weighted in a weighing paper and wrapped. The tester was occupied into an abstraction protector and shifted to a Soxhlet. A 250 ml of petroleum ether was added in the flask and it was taken on the warming mantle, which was connected the Soxhlet apparatus, turned on the water bath to cool them. The sample was excited for 14 h (The heat rate of 150 drop/min), and the flush was vaporized using the vacuum condenser. The flask was then incubated at 80–90 °C until the flush was fully vaporized, and the flask was utterly dried. The flask and its dehydrated content were reweighted. The fat content was calculated by the following Eq. (5).

$$\text{Fat (\%)} = \text{Weight of fat} * 100/ \text{Weight of sample} \quad (5)$$

2.15.4. Crude fiber content

Two-gram sample was weighted and taken in a 500 ml beaker. 200 ml of hot H_2SO_4 (0.125M) was supplemented to it and incubated approximately 30min. The beaker was placed under a condenser and boiled for 30 min. The residue was transferred back to the beaker. Then added 200 ml hot NaOH (0.313M) and it was boiled for 30 min by placing it in the condenser. The content was filtered and washed with boiling water supplemented with 1% HCl. The content was dried overnight at 100 °C, then cool and weighted. The crude fiber content was calculated by the following Eq. (6).

$$\text{Crude fiber content (\%)} = (W_2– W_1) *100/W \quad (6)$$

2.16. Determination of iron and zinc micronutrients in maize

The harvested leaves and grain were splashed in $CaSO_4$ (1mM) for 5min. Fleshy tissue samples were spalhed with deionized H_2O at 3 repeated times prior to drying at 80 °C for 2 days in a drying stove. Afterwards, fleshy tissue was digested with 5 ml HNO_3 and 2 ml $HClO_4$ in a glass beaker and excited in a microwave oven for 3min. The Atomic Absorption Spectroscopy (AAS) (Model No. AA-6800, Shimadzu, Kyoto, Japan) was used for the estimation of Fe and Zn micronutrients.

2.17. Statistical analysis

The experiment was assembled on a entirely randomized block design (CRBD) where, 3 replications contain for every single treatment of the sample. The significancy of every single group data was evaluated by Microsoft Excel and at a significance level of $P \leq 0.05$ by one-way ANOVA, which was tracked by Duncan's Multiple Range Test (DMRT) in SPSS Statistical 20 software. GraphPad Prism-8 was used for graphical presentation.

3. Results

3.1. Plasma species identification

The emission spectrum was measured with a spectrometer (Ocean Optics: USB2000 + XR1, slit length: 25μm, grating 800 $lines/mm$) was shown in Figure 2. Maize seeds to be treated with LFGD plasma where, treatment durations were 30s, 60s, 90s and 120s. The species produced in the Ar and the O_2 discharge are: $N_2(C^3\Pi_u - B^3\Pi_g)$ in the range 294 – 380 nm, $OH(A - X)$ in the range 306–312nm, first negative system $N_2^+(B^2\sum_u^+ - X^2\sum_g^+)$ in the range 391 – 405 nm, atomic oxygen, and argon lines range in 737.21–810.37nm. A trace amount of nitrogen species is like to be present in the discharge.

3.2. Maize seed surface modifications

In Figure 3 represents the micrographs of control and the 90 s LFGD (Ar + O_2) plasma treated maize seed surfaces which shows explicit

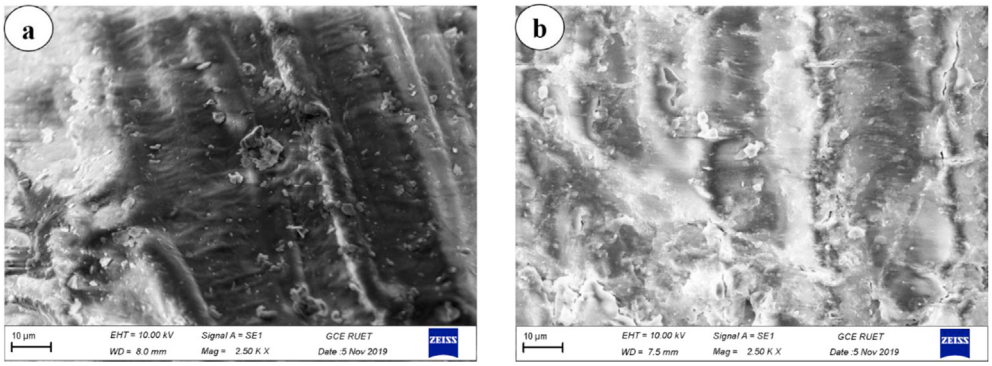

Figure 3. SEM imageries of the surfaces of maize seeds where, (a) the control and (b) the 90 s treatment with LFGD (Ar + O_2) plasma. Scale bar is 10µm, Magnification 2.50 KX.

changes on the surface structure of the maize seeds upon treatment. The control seed surface presents a waxy layer and the structure of its surface smooth. SEM analyses of these two figures specify that the surface of the seeds become rough when treated for the 90 s using LFGD plasma compared to control.

3.3. Germination rate

In the current experiment the highest seed germination rate of the control and 90s LFGD (Ar + O_2) plasma treated the seeds were found to be 75.81 ± 1.47% and 91.71 ± 1.11% respectively. So, the germination rate of LFGD plasma treated seeds increased up to 15.88% with the escalation of treatment interval up to 90s, which is greater than the control. The results shown in (Figure 4). However, treatment for a longer time 120s causes the decline of the germination rate.

3.4. Morphological and the physiological properties of maize plants

Maize plants fully-fledged from LFGD (Ar + O_2) plasma treated the seeds showed significant changed in different morphological and physiological characters, *viz.* shoot length, root lengths, the number of leaves, stem diameter, the fresh weight and the dry weight related to control were shown in Table 1. Among all these conducts, LFGD (Ar + O_2) plasma yielded the highest shoot lengths (33.42 ± 28.49%), root lengths (10.67 ± 0.95%), the number of leaves (8.34 ± .91%), the stem diameter (13.37 ± 0.03%), fresh weight (55.33 ± 3.85%) and dry weight (26 ± 1.57%) respectively compared to control as shown in Table 1. All these increments were found to be statistically significant.

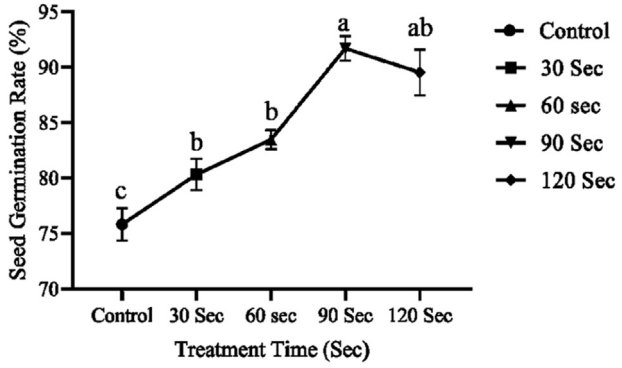

Figure 4. Effects on the germination rate of maize seeds treated by LFGD (Ar + O_2) plasma. This graph represents the mean treatment of 3 replications and the capped appearances represent the standard error of the mean.

3.5. Determination of chlorophyll concentration

The total concentrations of chlorophyll in leaves were improved in all plants grown-up from plasma treated maize seeds compared to controls. The uppermost chlorophyll concentration is 46.93 ± 2.78 mg g^{-1} twisted in the leaves grown-up from the maize seeds alleviated for the 90sec. Figure 5 appearances the total chlorophyll (a and b) concentrations of the maize plants fully-grown from control seeds and LFGD (Ar + O_2) plasma treated seeds.

3.6. The hydrogen peroxide and the nitric oxide concentrations

The H_2O_2 and the NO concentrations were found in leaves and in roots of the plants fully-fledged from the desire maize seeds treated with LFGD (Ar + O_2) plasma in contrast to control. The highest concentration of H_2O_2 was found to be 208.33 ± 5.87µ $molg^{-1}$ FW in the leaves [Figure 6(a)] and 61.13 ± 1.72µ $molg^{-1}$ FW in the roots [Figure 6(b)] in the plants grown from 90 s plasma treated seeds. On the other hand, the highest concentrations of NO were 369.24 ± 213.19µ $molg^{-1}$FW in the leaves and 1094.23 ± 135.44µ $molg^{-1}$FW in the roots respectively, where the seed treatment duration was 90 s [Figure 6(c) and (d)]. Both H_2O_2 and NO concentrations irrespective of leaves and roots were increased at a significant level in respect to control.

3.7. The total soluble sugar, protein and phenol concentrations

The total soluble sugar (TSS) concentrations were increased significantly compared to control when seeds treatment durations were 30sec, 60sec, 90sec, and 120sec of LFGD plasma. The maximum concentrations of TSS were found in leaves 1.62 ± 0.19µ mol g^{-1}FW [Figure 7(a)] and 2.89 ± 0.81µ mol g^{-1}FW in roots [Figure 7(b)] where, the treatment seeds duration was 90 s. The total soluble protein (TSP) in leaves and roots was correspondingly amplified with different treatment duration. The maximum concentration of TSP in leaves was observed 52.48 ± 1.69µ mol g^{-1} FW [Figure 7(c)] for the seed treatment duration in 90 s and 76.03 ± 1.26µ mol g^{-1}FW [Figure 7(d)] in roots respectively, the treatment duration was 120 s. On the other hand, the highest concentration of total soluble phenol was 21.68 ± 1.45µ mol g^{-1} FW [Figure 7(e)] in leaves, and 51.54 ± 0.95µ mol g^{-1} FW [Figure 7(f)] in roots for seed treatment duration 90 s in both the cases.

3.8. Investigation of antioxidant (CAT, SOD, APX and GR) enzymes

Concentrations of CAT, SOD, APX, and GR were investigated in the leaves and roots of the maize seeds treated with LFGD (Ar + O_2) plasma in four different timings to compare with those of control. Results are shown in (Figure 8). The highest CAT concentration was found in both leaves 8.72 ± 0.37µ mol g^{-1} FW [Figure 8(a)] and roots 6.83 ± 0.44µ mol

Table 1. Morphological and physiological properties of maize plants fully-fledged from the seeds treated with LFGD (Ar + O_2) plasma. Different letters in this table signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

Features	Shoot length (cm)	Root length (cm)	Number of Leaves	Stem diameter (mm)	Fresh weight (gm)	Dry weight (gm)
Control	94.29 ± 4.74^{c}	16.00 ± 2.1^{a}	9.77 ± 2.27^{c}	21.24 ± 4.04^{b}	140.00 ± 8.16^{ab}	32.66 ± 5.03^{b}
30 Sec	106.65 ± 17.57^{bc}	23.66 ± 5.50^{a}	10.77 ± 1.92^{b}	23.81 ± 3.95^{ab}	143.33 ± 18.14^{ab}	25.33 ± 4.16^{c}
60 Sec	116.12 ± 36.06^{ab}	25.33 ± 12.05^{a}	11.00 ± 0.70^{b}	27.55 ± 4.93^{ab}	135.33 ± 16.01^{b}	34.00 ± 3.46^{b}
90 Sec	127.71 ± 33.23^{a}	26.67 ± 1.15^{a}	18.11 ± 1.36^{a}	34.61 ± 4.01^{a}	195.33 ± 12.01^{a}	58.66 ± 3.46^{a}
120 Sec	122.43 ± 29.45^{ab}	24.00 ± 3.46^{a}	12.66 ± 2.29^{ab}	28.33 ± 2.77^{ab}	110.00 ± 5.38^{c}	31.33 ± 1.52^{b}

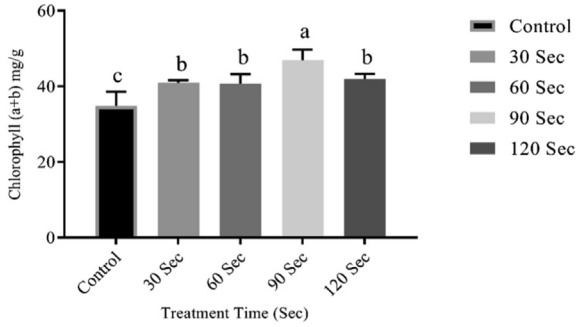

Figure 5. The total chlorophyll concentrations (a and b) of the leaves in response to LFGD (Ar + O_2) plasma treatment on seeds. Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

g^{-1} FW [Figure 8(b)] for maize seeds conductive extent found in 90sec. The maximum concentrations of SOD were observed in leaves was 6.28 ± 0.59μ mol g^{-1} FW [Figure 8(c)] used for 90sec seed treatment and 4.75 ± 0.35μ mol g^{-1} FW [Figure 8(d)] in root respectively, the treatment duration was 120sec. The highest APX concentration was 5.56 ± 0.24μ mol g^{-1}FW [Figure 8(e)] found in leaves and 7.91 ± 0.34μ mol g^{-1} FW [Figure 8(f)] the roots respectively, provided by maize plants fully-grown after the seeds treated using LFGD (Ar + O_2) plasma and the treatment duration in both was 90sec. In case of GR concentration, there were no significant changes were perceived in leaves [Figure 8(g)] of maize plants propagated from seeds treated by means of LFGD (Ar + O_2) plasma and interconnected to control. The maximum GR value was perceived 0.19 ± 0.01 μmol g^{-1}FW [Figure 8(h)] in roots of the maize plants respectively. Where, the treatment duration was 90sec.

3.9. Yield of the maize

The average number of grains was counted and the average weight of grains per cob was taken to check the variation in the yield in response to plasma treatment. Both the agronomic traits were improved due to exposure of LFGD (Ar + O_2) plasma treated maize plants compared to control. The maximum average number of grains (483.33 ± 15.60) and the maximum average weight of grains (342.00 ± 2.35 gm) were obtained from LFGD plasma; the treatment time duration was 90sec that shows significantly increased with respect to control [Figure 9(a), (b)]. However, the almost equally increased weight of grains per cob (340 ± 13.37 gm) were obtained from the plants grown from 30 s of LFGD (Ar + O_2) plasma treated seeds.

3.10. Determination of nutritional compositions (moisture, ash, fat and crude fiber)

To check whether or not there are any qualitative changes in the nutritional compositions the maize grains collected after the plants fully-fledged from the plasma cured seeds in contrast to control. However, no substantial fluctuations were perceived in the composition of nutritional values such as; the moisture content, ash content, fat content and crude fiber content in the grains of control and LFGD (Ar + O_2) plasma treated groups [Figure 10 (a–d)].

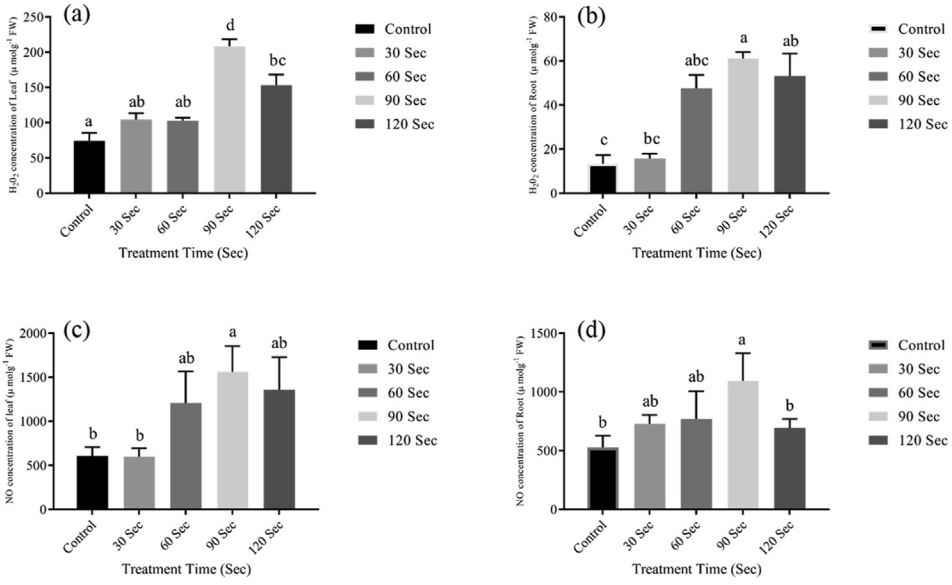

Figure 6. The change in hydrogen peroxide (H_2O_2) and nitric oxide (NO) concentrations leaves and roots of maize plants treated with 90sec LFGD (Ar + O_2) plasma. (a) H_2O_2 concentration in leaves and (b) roots, and the NO concentrations in (c) leaves and (d) roots of LFGD (Ar + O_2) plasma treatment. Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

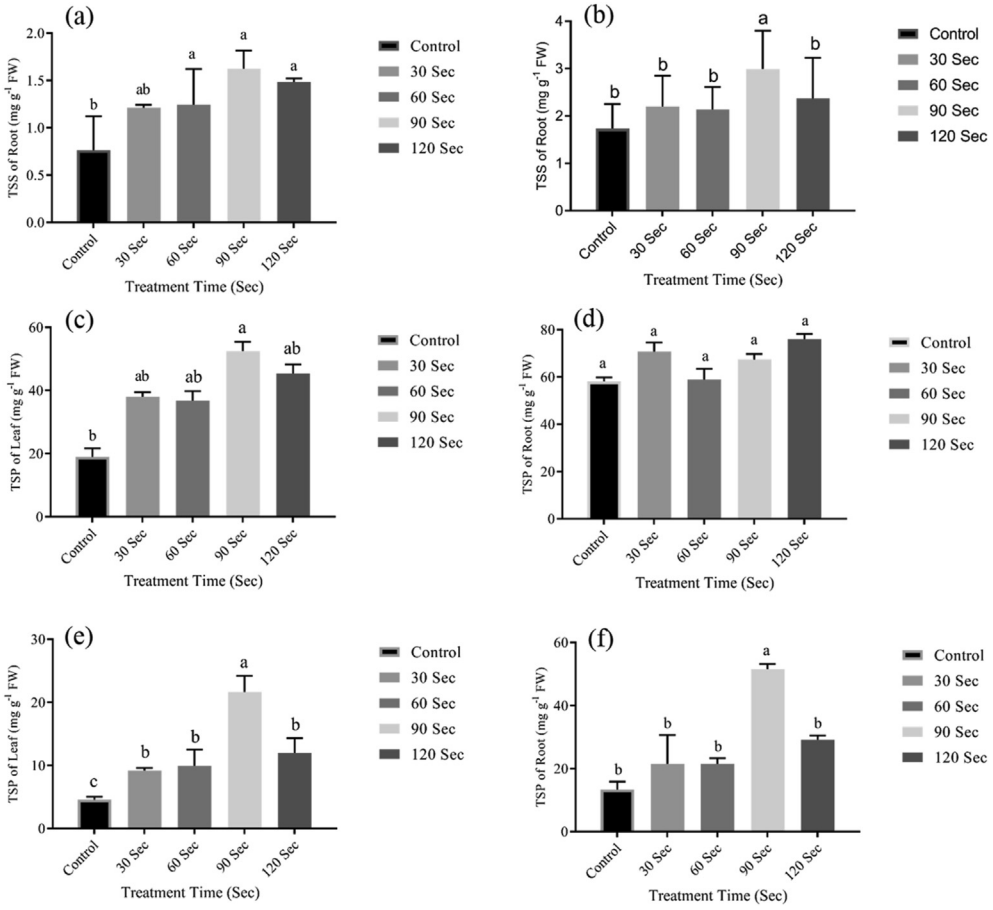

Figure 7. Changes in TSS, TSP and TSP concentrations in laves and roots of plants grown-up from 90sec LFGD (Ar + O2) plasma treated the seeds of maize. (a) TSS concentration in leaves and, (b) roots, and TSP concentration in (c) leaves and, (d) roots and TSP concentration in (e) leaves and, (f) roots of LFGD (Ar + O_2) plasma treatment. Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

3.11. Determination of iron and zinc micronutrients in maize

Atomic Absorption Spectroscopy (AAS) exposed that Fe concentrations enticingly improved both in grains and leaves of maize. The maize plants instigated after the seeds treated by means of LFGD plasma treatment associated to non-treated control. The maximum concentration was observed in grains 51.85 ± 8.45 mg g$-^{1}$DW [Figure 11(a)] and in leaves 19.83 ± 6.046 mg g$-^{1}$DW [Figure 11(b)] respectively, treatment time was 90 s in both the cases.

In addition, Zn concentrations significantly improved both in grains and leaves of maize. The maximum concentration of Zn was observed in grains 13.46 ± 3.44 mg g$-^{1}$DW [Figure 11 (c)] and in leaves 12.86 ± 0.32 mg g$-^{1}$DW [Figure 11 (d)] respectively, treatment time in both the cases was 90 s.

4. Discussion

The fast-growing world population requires more food for survival. For this reason, we need to introduce some new technology in our conventional agricultural system that will help to overcome it. Plasma treatment is a innovative tools that exhibited its usefulness for the enhancement of agronomic individualities of diverse crops [28]. The outer coat of most of the cereal grains contains a waxy layer. When plasma treatment is applied to the seeds, the seed surface becomes rough and skinny as related to the seed skin of control. The possible mechanism of the effect of plasma treatment on seed is explained by the increase in temperature (~40 °C) [29]. When energy was released by mean as high temperature through the plasma reactor the wax and consequently the waxy coat was vaporized, and it also reacted with RNS and ROS species such as; NO, NO_2, N_2O, HNO_2, HNO_3 laterally numerous extremely reactive oxygen and OH are produced which react to the surface of the seeds as well as the seeds coat turn into thin and rough. The thin seed surface and the roughness of the seed exterior are responsible for faster water uptake and the enhancement of the nitrogenous compound [18, 29, 30, 31]. The enhanced amount of reactive nitrogen and oxygen species along with transmembrane protein aquaporin (AQPs) on the cell surface is responsible for conducting water molecules [32, 33]. It was reported that for the better water absorption depends on the increased the nitrogenous molecule [18]. Nitrogen and phosphorous molecules can accelerate the growth of maize plants under water insufficiency conditions [34].

The exposure of maize seeds to LFGD (Ar + O_2) plasma showed stimulating effects on the germination rate, growth, and development. In our study, the exposure of the 90sec LFGD plasma may directly or indirectly be involved to accelerating the enzymatic activities which were related on seed germination and also accelerate the inner decomposition of the essential nutrients which trigger those compounds to increase the utilization of seed reserved and the growth of seedlings. It was examined that, DBD (dielectric barrier discharge) plasma treatment have the potential ability to improving the seed germination rate and vigor index in wheat plants [35, 36, 37].

In this study, modifications between the treated and non-treated seeds in their functionalities were openly perceptible through the complete research. In this study LFGD plasma treatments produced diverse functional reactive nitrogen and oxygen species. Among them, the oxygen having functional groups of plasma species enrich the exterior coat of seeds that consequence trendy an essential progress on wettability and lastly enhanced the seed germination rate [29, 38]. For this reason, the interaction of RNS and ROS generated through plasma treatment on the maize seed coat which might enhances the seed germination rate.

According to [39] Dobrynin et al., the generated plasma which interact with the surface of the seeds resulting the enhancement of seed

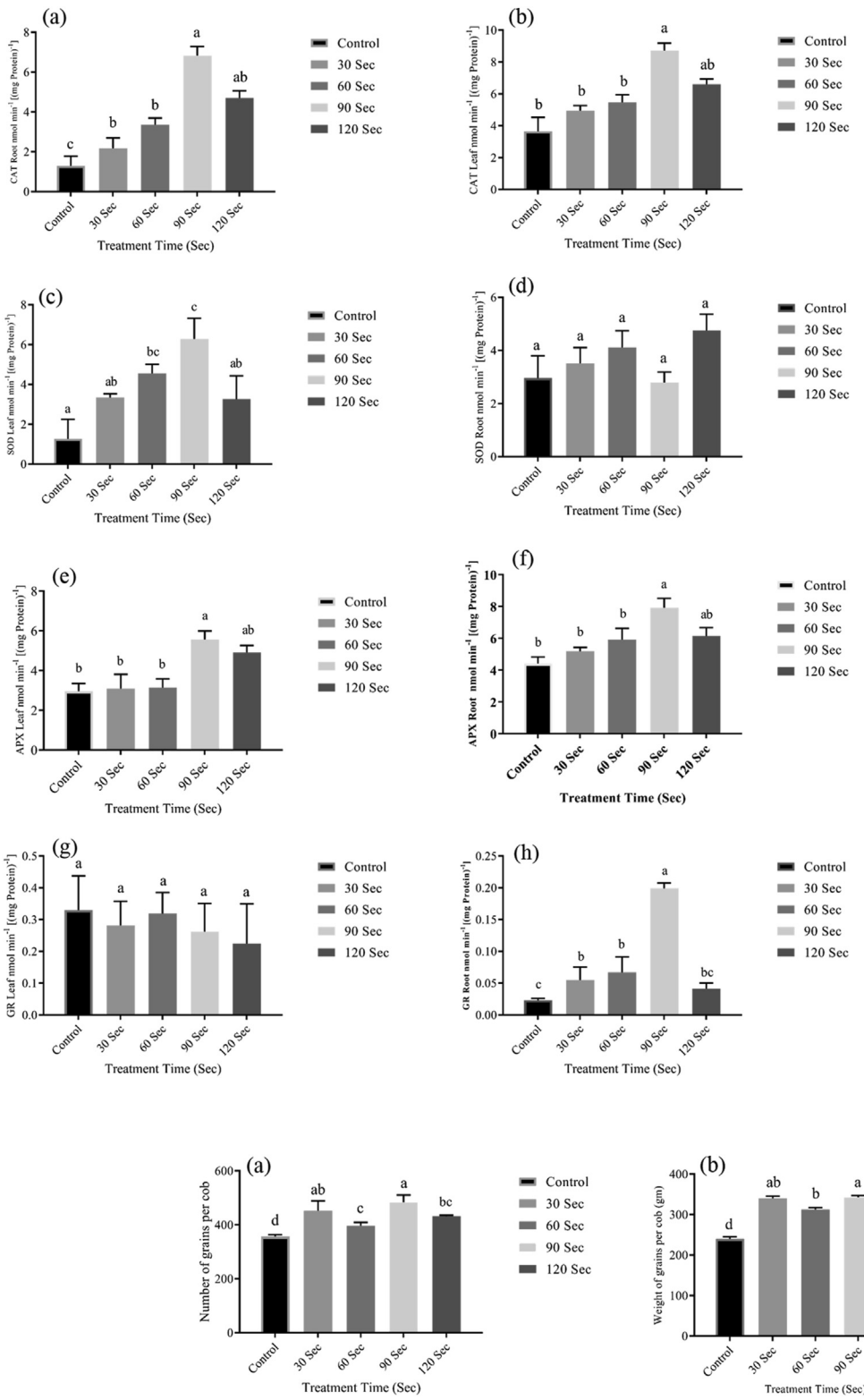

Figure 8. Changes in CAT, SOD and APX concentrations in laves and roots of maize plants grown-up after 90sec LFGD (Ar + O_2) plasma treated the seeds of maize. CAT concentrations in (a) leaves and (b) roots; SOD concentrations in (c) leaves and (d) roots; APX concentrations in (e) leaves and (f) roots; and the GR concentrations in (g) leaves and (h) roots. Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

Figure 9. The graphs represent (a) the average number of grains and (b) the average weight of grains per cob of maize plants grown-up from the maize seeds treated by means of LFGD (Ar + O_2) plasma treatment. Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

germination rate as well as accelerate the enzymetic activity. That's why the inner essential nutrients of the seeds decomposed which contribute to utilized the seeds reservior and increasing the growth of microplants. From our study, it was established that LFGD (Ar + O_2) plasma usage initiated substantial changes in shoots length, roots length of maize plants and also exposed a considerable progress in fresh and dry weight both in shoots and roots.

In the current project, it was created that the total chlorophyll concentrations through the LFGD plasma treatment to the maize seeds were expressively higher than the non-treated seeds. Previously, it was

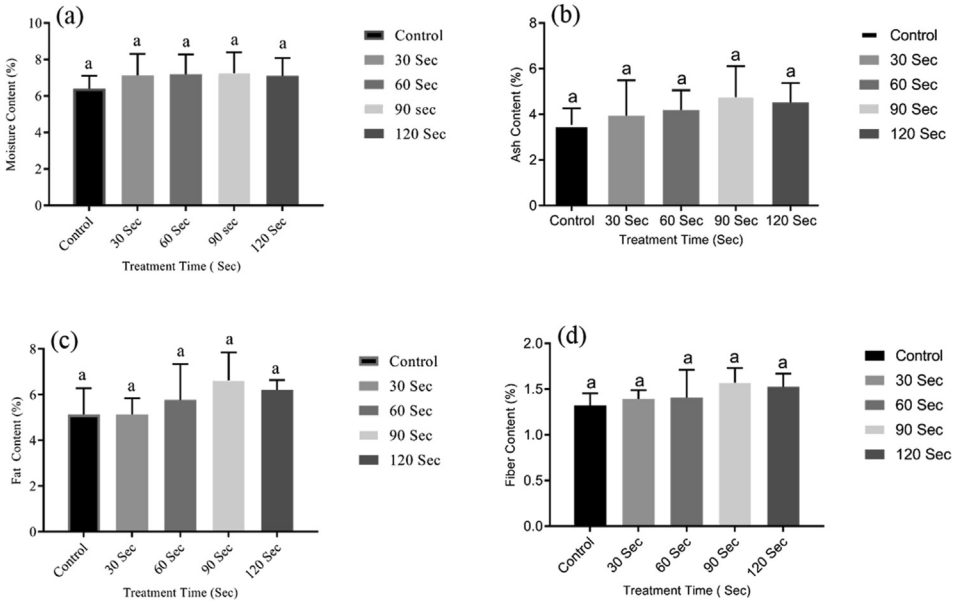

Figure 10. The effects of LFGD (Ar + O_2) plasma treatment on the Nutritional compositions of control and treated groups; The (a) moisture content, (b) ash content, (c) fat content, and (d) crude fiber content of maize. Where, maize plants grown-up from the seeds treated by means of LFGD (Ar + O_2) plasma treatment. Letters in the figure represent Duncan's Multiple Range Test (DMRT). Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

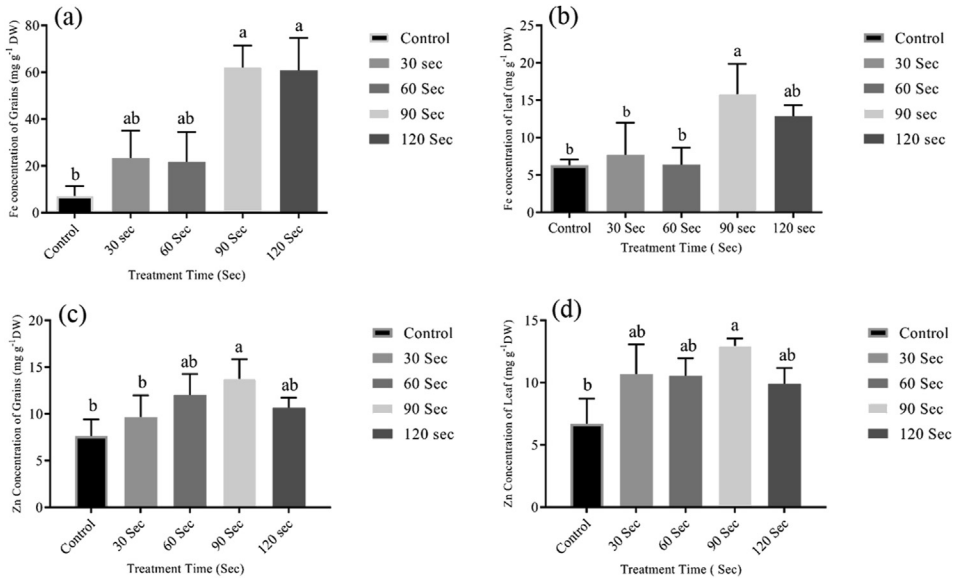

Figure 11. The effects of LFGD (Ar + O_2) plasma treatment on the iron and zinc concentration in the grain and leaves in control and treated groups. Concentration of Fe and Zn was measured using Atomic Absorption Spectrophotometer. (a) concentration of Fe in grains (b) concentration of Fe in leaves (c) concentration of Zn in grains and (d) concentration of Zn in leaves. Different letters in this figure signified by Duncan's Multiple Range Test (DMRT). Similar and dissimilar letters are contrasted that they are significantly different as per DMRT at $P < 0.05$ significant level.

examined by two scientists [6, 40] that plasma treatment have the potential ability which might enrich the physiological activity of the plant. Chlorophyll concentration in leaves significantly increased the presence of a sufficient amount of nitrogen content [41]. Chlorophyll is the green pigment in the leaves of a plant. The quantity of chlorophyll pigment is an indicator for photosynthetic capacity of plants and also indicates the nutrient availability. Due to the application of LFGD plasma, which produces reactive nitrogen species, it assists as a key foundation of nitrogenous compound. For the made of chlorophyll pigments nitrogenous compounds required which play a dynamic character for the supplementation of chlorophyll concentrations in leaves and LFGD plasma produced and induced the source of nitrogen.

In the present study, the 90sec LFGD (Ar + O_2) plasma treatment can accumulate the soluble sugars and proteins in maize plants from seeds to plantlets, which might explanation for the increased growth of maize plants from treated groups. The soluble sugar, which is also known as the photosynthetic outcome of plants, which is the major constituents for the metabolic pathway of plants and it is strictly correlated to the mechanisms of photosynthesis and yield [42, 43]. Besides, the soluble protein which is an identical component of several plant enzymes, plays a significant impact on growing plants and redirects the entire metabolic pathway of plants [44]. Previously, observed that the total soluble sugars and proteins increased in soybean and brown rice through the application of cold plasma treatment [45]. Also reported the inductions of cold plasma to the seeds, and it enhanced the seed germination, seeding growth, antioxidants, drought stress tolerance potentiality and the defense gene expression of the tomato [46].

In this study, the concentration of hydrogen peroxide and NO involve to increased in the germinated maize plants due to plasma treatment. Hydrogen peroxide is considered as an anxiety element in plants. In case of the enhancement of the soluble sugar compounds in leaves both hydrogenperoxide and nitricoxide plays a significant role in signal transduction pathway. Although H_2O_2 is measured as a signaling molecule, it helps to break down seed dormancy, germination, and

antioxidant defense [47]. Another bioactive signaling molecule, nitric oxide (NO) participates in control and guideline in many phases of plants growth [47]. For the breakdown of seeds latency and enhance the germination rate NO plays a critical role [47], our current study report is supporting these findings.

CAT, SOD, APX, and GR are mainly active against higher H_2O_2 concentrations. These enzymatic records were interconnected with the seed's hydrogen peroxide concentrations cutting-edge the logic that CAT is unique and only the key antioxidant enzyme connected by the way of H_2O_2 sifting. Hydrogen peroxide is despoiled by the CAT enzyme in an energy-efficient method, which consequences from the manifestation of the *VmCAT* gene [48]. The SOD is known as a principal mediator which produce ROS to protect plants in contrast to the oxidative stress. The SOD enzyme is a type metalloenzymes, which act as a dismutase property on superoxide radicals to H_2O_2 and O_2 [38]. The APX enzymatic activity, both in leaves and roots, was amplified expressively due to the application of plasma treatment. The APX concentrations in the maize plants might be related to the ASH-GSH (Ascorbate glutathione) cycle, which plays a vital role to detoxify the H_2O_2 molecule and protect plants from stress conditions. Glutathione reductase (GR) is an enzyme that plays an important role in both biotic and abiotic stresses in plants. It was observed in several plant species like rice, wheat, barley, and maize, the GR activity is increased in roots and shoot tissues. The increased amount of GR plays an important role in Fe homeostasis in gramineous plants and thereby allows plants to cope with Fe deficiency [49].

The utilization of Fe is in need of the plant's capability to convert Fe^{3+} to the Fe^{2+} [50]. It controls that LFGD ($Ar + O_2$) plasma treatment can also change the electron discharge, which accelerates the iron uptake mechanisms. For the estimation on maize-dependent countries, maize provides more than 20% of dietary protein. Hence, maize is an attractive food for Fe bio-fortification, dietary intakes and usual eating patterns related to ID and anemia [51]. Another micronutrient Zn plays a vital role in a plant's nitrogen metabolism in plants; as a result, it improves protein quality; it also plays a key role in photosynthesis [52]. The high concentrations of Zn, B and Mo micronutrients will help to increase the maize seed germination, emergence and stand establishment [53]. In such a way, Zn is effective in increasing production [54]. The increased amount of Zn in leaves of the plants also helps to increase starch percent in maize during harvest [55]. Nowadays plasma technology is effective tools for the enzymatic extraction of arsenic and selenium ion from rice flour [56].

The compositions of moisture, fat, crude fiber, and ash content maize is crucial that are good sources of essential nutrients required for proper growth and development both in humans and animals. The most important component of the maize kernel was used for the production of snacks is the endosperm, which contains high content of starch and protein [57]. The percentage of moisture in maize in this study ranged from 7.1% to 7.23%. However, the reported moisture content maize is 7.16% [58]. It was observed that [59] if the moisture content is below the desired range, the grain might be at risk to attack different diseases.

Fat is a principal constituent in maize grains. The fat content in seeds plays a vital role in keeping good human health as they act as a vehicle for fat-soluble vitamins [60]. The percentage of the fat content in maize seed was increased by 6.633% at 90sec LFGD plasma treatment with respect to control grain by 5.133%. Okonkwo & Agharandu et al., found the fat content in maize ranges from 4.07% by Adeyeye & Ajewole et al., reported [61] the effect of germination of maize through the presence of nutrient and anti-nutrient compositions.

The plant fiber is composed of cellulose and hemicellulose, which is very good for human metabolism as it increases water retention capacity during the passage of food along the gut. The percentage of crude fiber contents in maize was found in the range 1.39%–1.57% which is marginally higher than the informed values 0.95%. In our study, the ash content of seeds is significantly enhanced by 3.94%–4.756% [62, 63, 64] in comparison to the ash content 1.10–2.95% from noncommercial yellow maize flour.

5. Conclusion

Obtaining high yields in agricultural production is vital to mitigate the increasing food demand for the rapidly growing world population. 'Plasma Agriculture' is an unindustrialized interdisciplinary sector of plasma applications. It is required to support maintainable expansion and compensate for losses of food and agricultural manufacture to certify adequate production for upcoming generations. Maize is the most advanced significant cultivated crops throughout the world including Bangladesh. This comprehensive study reveals that the application of LFGD plasma technology for enhancement of the seed germination, plant growth, productivity and nutritional composition in maize.

In this study, it was revealed that 90 s LFGD plasma treatment maize seeds could improve morphological and physiological characteristics, which were achieved through the tight regulation of antioxidant enzyme CAT, SOD, APX, GR and signaling of H_2O_2 originated from the maize plant due to plasma treatment. LFGD plasma treatment may be frequently used to overcome the low yield and results in the loss of soil fertility. The conclusions of this research work will be convenient to optimize LFGD plasma technology in maize and other crops with an observation to improve agronomic properties.

Message for reader

From this manuscript, the reader can easily access the information about the LFGD plasma generation through two combinational gases (argon and oxygen) and their optimum concentrations. To know the generated plasma has the potential impact on maize seeds which enhance the overall agronomic characteristics such as the seed surface to productivity as well as to change the conventional cultivation systems.

Declarations

Author contribution statement

Sumon Karmakar; Mutasim Billah: Performed the major experiments; Analyzed and interpreted the data; Wrote the paper.

Mahedi Hasan; Sohanur Rahman Sohan; Md. Forhad Hossain: Performed a few experiments.

Md. Mamunur Rashid: Analyzed and interpreted the data.

Mamunur Rashid Talukder; Kazi Md. Faisal Hoque; Ahmad Humayan Kabir; Md Abu Reza: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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