Plasma Chemistry and Plasma Processing (2019) 39:897–912
https://doi.org/10.1007/s11090-019-09969-6

ORIGINAL PAPER

Cold Atmospheric Pressure Nitrogen Plasma Jet for Enhancement Germination of Wheat Seeds

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Received: 21 October 2018 / Accepted: 21 February 2019 / Published online: 2 March 2019

Abstract

The possibility to improve the germination characterization of the wheat seeds by cold atmospheric nitrogen plasma jet treatment was report. Spectroscopic measurements were performed to identify the constituent particles of a nitrogen atmospheric plasma jet. Spectroscopic measurements revealed that the particles with relatively high energy excited states exist inside the jet nozzle. During this study the cold atmospheric plasma parameters were set for attain the highest rate of the germination seeds. The nitrogen used in this system as carrier gas. The nitrogen cold atmospheric plasma operated at 14 l/min as a fixed flow rate. The operation of plasma jet at 14 l/min was represented the suitable plasma dose to enhancement the germination parameters. The N_2 first positive system (N_2 1+), N_2 second positive system (N_2 2+) and N_2 ion first negative system (N_2^+ 1−) were represented the emission spectra observed inside the jet nozzle. The germination characteristics of wheat seeds have been significantly enhanced after cold atmospheric plasma treatment. The mean weight of fresh sprout was 823.82 mg for untreated seeds and increased to 1231.80, 1369.50 and 1342.46 mg for 2 min, 4 min, and 6 min treatments respectively. The effects of nitrogen cold atmospheric plasma jet on the growth parameter depended on exposure time.

Keywords Plasma jet · Spectroscopic measurements · Germination rate · Seedling growth

Introduction

In the last two decades non-equilibrium atmospheric plasma, also known as cold atmospheric plasma (CAP), has undeniably risen an increasingly amount of interest both in the scientific and industrial worlds. While it's potential is still to be fully discovered, its role as one of the leading scientific innovations of the twenty-first century is less and less

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questioned. However, because the facility of non-equilibrium atmospheric plasma devices design as well as their low cost, recently it has been using in many applications. Among the different types of non- plasma, the cold atmospheric plasma jet (CAPJ) represented a promising sort in the treatment of surfaces and living tissues [1–6]. In plasma technology the term *plasma jet* is used to refer to many different configurations and types of plasmas, having in common that the gas discharge is operated in an open volume electrodes arrangement and plasma is projected outside the electrode arrangement into the environment. The expansion of the plasma is often due to a high flow of a working gas, usually a noble gas, flown through a capillary before expansion into ambient air, where it forms a channel, also named plume, surrounded by air. The important advantages of cold atmospheric plasma jet is the running it in open air because it overcomes the obstacles represented by the use of vacuum-based plasma. An important advantage of CAPJ is the ability to generating certain chemical compounds that can penetrate the treated material surface [7]. Over the past years, the advantages of many CAPJ devices have been studied also the applicability in the medical and biological fields have been investigated [8–16].

Recently, plasma has attracted the attention of many researchers, especially in the field of energy transformations and biological sciences. Plasma treatment can be applied efficiently when controlling plasma parameters such as the appropriate reactive species, Species density, plasma temperature and plasma dose, which can stimulate and accelerate biological processes [17]. As the plasma produced ions, electrons and excited species interact with ambient air, other RONS are generated. The RONS produced in this manner are believed to be the main agents in biomedical applications including atomic oxygen (O), hydroxyl radicals (OH), ozone (O_3), hydrogen peroxide (H_2O_2) and nitric oxide (NO) [18].

In the agricultural applications, researchers are interested in the interaction between the reactive species which generated by cold plasma and the seeds, which would improve many properties, including the removal of contamination of seeds [19, 20], water uptake imbibation [20], breaking of dormancy [21], enhancement of seed germination [22–24] and seedling growth [25–29], and draught stress [30]. Cold atmospheric plasma which consisting of electrons, molecules, excited atoms, ionized gases, radicals and strong electric field considered as one of the physical methods for seed treatment. Previous studies have demonstrated that cold plasma treatment could enhance seed germination and seedling growth of many plants including Carthamus tinctorium L., wheat, tomato, soybean [31–33], and watermelon [34].

A hydrophobic and a hydrophilic thin layer can be formed on the surface of the treated material after plasma treatment. This change of the material surface gives positive results with respect to different cultivation conditions and seed types. Volin et al have studied the effect of hydrophobic thin layers on the delaying imbibition in seeds sown in the different types of the soil [35]. it is well known that the seeds with high moisture content can be susceptible to imbibition chilling injury.

So that, the hydrophobic thin layer has a powerful effect on the improving viability in some seeds which occur by reducing water uptake [36–39]. On the other study, by improvement the hydrophilicity of seeds this led to enhancement the water uptake and the seeds germination [29]. Previous studies have shown that plasma has high sterilization efficiency for seeds and preserved foods because of the inactivation occur for many microorganisms through this method [40, 41]. The effect of cold atmospheric plasma on various food contaminant microorganisms such as, *A. flavus*, *Staphylococcus aureus*, *Fusarium* spp., *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger*, *Escherichia coli*, *Bacillus subtilis* and other microorganisms have been studied on different materials [42–44]. Plasma used to inactivation of many food contaminant microorganisms like, *Fusarium* spp., *A.*

flavus, *Aspergillus niger*, *Candida albicans*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Bacillus subtilis*, Escherichia coli, and other microorganisms have been studied on different materials [42–44].

Wheat is the essential strategic crop for one-third of the world's population [45]. As for Egypt, it is considered as the first strategic crop as it represents the significant part of the Egyptian diet. Currently, there is a significant insufficiency of wheat production in Egypt, which leads to the import of a large proportion to overcome for this shortfall in production [46]. Because of the continuous increase in the population in Egypt, so we must find the appropriate ways to increase the production of wheat to overcome the extreme demand for food. The food needs of Egypt's population can be met by increasing seed germination rate as well as wheat plant growth. Recently we designed and constructed a nitrogen-plasma jet device designed; the device operated using a commercially neon power supply [16]. In this study, we focused on exploring the feasibility of nitrogen cold atmospheric plasma jet (NCAPJ) on seeds germination and seedling growth of wheat. The authors also investigate the optimization of the operating conditions in cold atmospheric plasma to increase the percentage germination and total vigor of seedlings.

Materials and Methods

Characteristics of Plasma Source

In the current study the NCAPJ system was used in the treatment of wheat seeds. As can be seen in Fig. 1, the design of the NCAPJ was described in an earlier study [13]. The high-voltage power supply is a commercially available transformer for neon light. The neon power supply is appropriated in the cold atmospheric plasma to produce the plasma jet to cut the overall cost of the system by substituting the expensive RF power supply representing the major cost. The output of the high-voltage neon power supply is 30 mA, 10 kV, and 20 kHz which lie in the range of very depressed frequency of RF. This power supply has an overload, open circuit, earth leakage and short circuit protection. The input of this power supply is connected to 220 V, 12 A voltage controller. The voltage controller regulates the primary voltage of the high-voltage transformer. The electrode system of the NCAPJ made

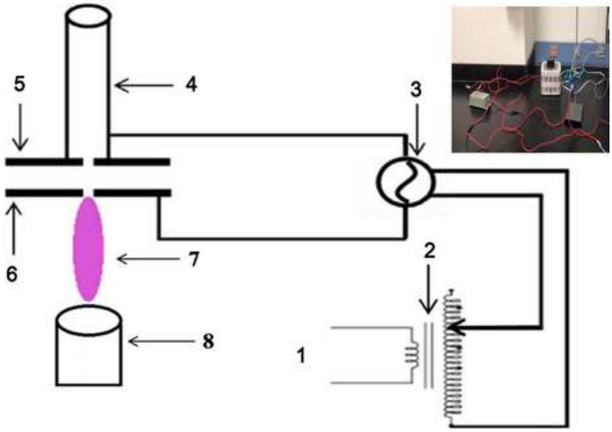


Fig. 1 The electric circuit of cold atmospheric plasma jet device, (1) input electric source 220 V, (2) voltage controllers, (3) neon power supply, (4) gas feeding through copper tube, (5) anode, (6) cathode, (7) plasma jet and (8) acrylic box. Attached photo display the NCAPJ system

up of two parallel stainless steel disks detached by Teflon insulator. The external electrode and the inside electrode have represented the cathode, and the anode respectively. However, the cathode and the anode have similar thickness and diameter 15 mm and 2 mm respectively, but the Teflon disk has a 1 mm thickness and 15 mm in diameter. The two electrodes and Teflon disk have center hole of 1 mm and 1.2 mm diameter respectively, through center hole nitrogen gas is flowing. The gas flow system is liable for transporting the gas to the plasma jet at the convenient flow-rate. It composed of the gas storage cylinder, binary-stage gas flow controller, and gas connection rubber hose. The Nitrogen was used in this work as a carrier gas. Once the nitrogen gas introduced through the two electrodes and the suitable high voltage applied between them, the nitrogen atoms are ionized by driving off electrons so that, the ionization of neighboring nitrogen species occurred by a collision with free electrons. After the series of these reactions the nitrogen gas converts to the plasma state.

Emission Spectrum and Discharge Voltage Measurements

An optical emission spectrometer detects photon emission in this region. Spectra obtained provide valuable information about species present within the plasma and their electron excitation, which in turn enables the calculation of vibrational and electron temperature within the plasma. The UV–visible emission spectra from the plasma reactor were obtained with a spectrometer which consists of a lens connected to a detector via fiber optic cable. In this work, the spectroscopy with the model number of HR4000CG-UV-NIR (Ocean Optics) was employed. However, the voltage measurement was performed using a high-voltage probe P6015 Tektronix HV probe and the currents by a TCP202 Tektronix current probe with a digital oscilloscope (Tektronix MSO4032).

Plant Material

The Giza 168 seeds one of the bread wheat cultivars were obtained from a private company. Healthy wheat seeds were selected and stored in plastic containers under the conditions of our laboratory at 50% relative humidity and temperature 24–25 °C.

Seeds Treatment

The plasma jet ejected to the acrylic box which contains wheat seeds. The wheat seeds were distributed regularly and then subjected to plasma radiation for different periods of time 2, 4, 6, 8 and 10 min. The distance between the nozzle of plasma jet and wheat seeds was fixed at 1.7 mm. In each treatment, 20 wheat seeds were unilaminar spread flat out on the bottom of the acrylic box; all procedures had been repeated four times. When the nitrogen flow rate was adjusted at 14 l/min and the 2.6 kV is the applied voltage, then the plasma jet generated between the two electrodes and ejected to the acrylic box. In this experimental, the length and the diameter of the plasma plume were 7 mm and 3 mm respectively, as can be seen in Fig. 2. The spatial uniformity of the plasma jet has been obtained at these conditions of the current system as can be seen in Fig. 2. However, the plasma temperature is one the most necessary parameters in biomedical applications attributable to the sensitivity of living cells and their inner organs like proteins. The plasma temperature should stay below an exact quantity of degree. During this method, plasma won't have any thermal effects like ablation or clotting. A

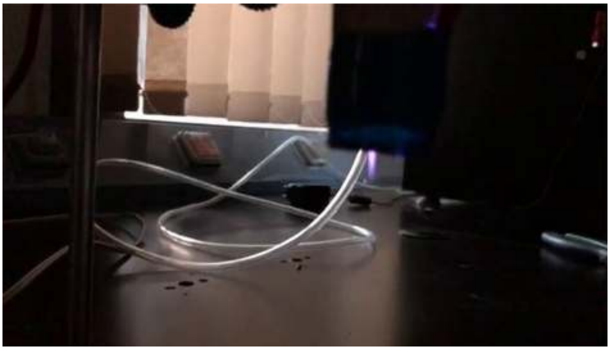


Fig. 2 Nitrogen cold atmospheric plasma plume

thermocouple was used to measure the plasma temperature. The gas temperature was varied from 25.5 to 31 °C when the period time of plasma radiation changes from 2 to 10 min.

One way ANOVA was used to analysis the obtained data and the comparisons with $P<0.05$ were considered significantly different. On the other hand, to measure the temperature of seeds through plasma treatment A GM1150 Infra-Red thermometer has been used. This thermometer has a measuring temperature range from −50 to 1150 °C.

Infrared thermometers measure the energy emitted by the surface focused onto the detector instead of surface temperature as such. The IRT detector is filtered to allow only a specific waveband, typically 8 to 14 km, onto the detector. This captured energy, E, is converted to temperature (T) via Stefan's Law, which states

$$E = \varepsilon\sigma T^4 \tag{1}$$

where ε is the emissivity of the object and σ the Stefan-Boltzman constant (5.68×10^{-8} J m^{-2} s^{-1} K^{-4}). Emissivity can be thought of as an "emittance efficiency factor"; Most plants have emissivity of 0.97 to 0.99 [47].

The wettability of treated and untreated seeds was calculated using the following equation:

$$\text{wettability} = \frac{m_1 - m_0}{m_0} \tag{2}$$

where m_0 represent the mass of seeds before soaked in water, and m_1 represent the mass of seeds after soaked in water. However, in current study the seeds were soaked for 12 h and weighted each 2 h after immersion in water.

Seeds were transferred to a transparent plastic cup containing one layer of filter paper, each cup contained twenty seeds. The Seeds germination process was took place in the dark at room temperature. The seeds germination process was observed for the duration of 10 days.

The following equations were used to calculate the germination characteristics of treated and untreated seeds [35].

After 24 h of cultivation, the germination potential was calculated using the following equation:

$$Germination\,potential\,\% = \frac{Number\ of\ \ seeds\ germinated\ after\ 1\ \text{day}}{Total\ number\ of\ seeds} \times 100. \tag{3}$$

However, the germination rate calculated after 4 days of cultivation from the following equation.

$$Germination\ rate\,\% = \frac{Number\ of\ seeds\ germinated\ after\ 4\ \text{days}}{Total\ number\ of\ seeds} \times 100. \tag{4}$$

Finally, the final germination percentage was calculated using the following equation after 10 days:

$$Final\,germination\,percentage\,\% = \frac{Number\,of\,germinated\,seeds\,after\,10\,\mathrm{days}}{Total\,number\,of\,seeds} \times 100 \tag{5}$$

Seedling Characteristics

After 10 days the seedlings were collected from all samples and shoot length was evaluated as the length of the seedling from the seed to the tip of the leaf blade. On the other hand the root length was evaluated as the length of the seedling from the seed to the tip of the root. Shoot and root fresh weight was evaluated as the weight of seedling shoot and root respectively, and expressed in milligram. However, Shoot and root dry weight was evaluated as the weight of seedling shoot and root after oven drying at 90 ºc for 48 h respectively, and expressed in milligram. Moreover the following equations were used to evaluate the seed vigor I and II [36]:

$$Vigor\,index\,I = \frac{fresh\,seedling\,weight\,in\,\mathrm{mg} \times germination\,percentage}{100} \tag{6}$$

$$Vigor\,index\,II = \frac{dry\,seedling\,weight\,in\,\mathrm{mg} \times germination\,percentage}{100}. \tag{7}$$

Result and Discussion

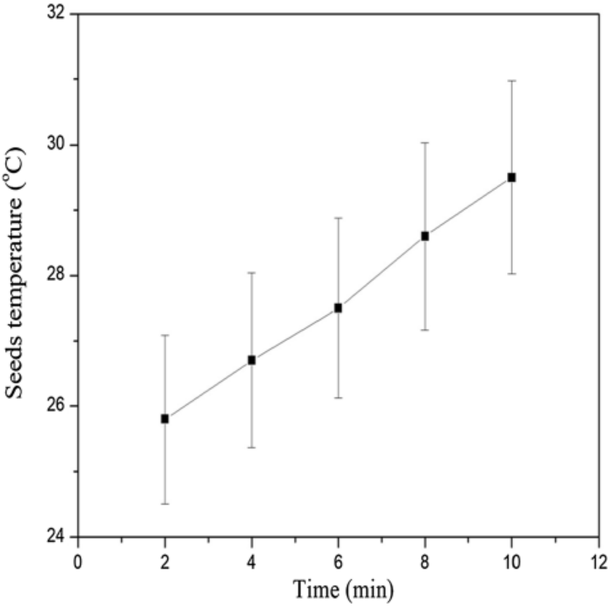
Seeds Temperature Measurement

Figure 3 shows the wheat seeds temperature through nitrogen plasma jet treatment using infra-red thermometer. From this figure, it can be showed that, by increasing the exposure time of plasma jet the temperature increase continuously. At 2 min of the operation time, the seeds temperature closed to room temperature (25.8 °C). Even the operation time reaches 10 min; the seeds temperature is as low as 29.5 °C. It means that, cold atmospheric plasma jet present a suitable method to enhancement germination without harmful effects.

Emission Spectrum and Discharge Voltage Measurements

Figure 4 shows the volt-ampere waveform of produced nitrogen plasma measured at 14 l/min as nitrogen flow rate. It can be seen from this figure that, the values of discharge voltage and discharge current is 2.6 kV and 38.1 mA respectively. However, the long plume plasma jet was observed by Ocean optical emission spectroscopy to investigate the behavior of atomic and molecular nitrogen and other constituent species. However nitrogen plasma emits high amounts of reactive species [48] so that; it used as a carrier gas in this experiment. The spectrum of the nitrogen plasma jet at flow rate of 14 l/m was showed in Figs. 5 and 6 from 200 to 500 nm and 600 to 900 nm respectively. The N_2 first positive system (N_2 1+), N_2 second positive system (N_2 2+) and N_2 ion first negative system (N_2^+ 1−) were represented the emission spectra observed inside the jet nozzle. The emission lines in Fig. 5 were primarily dominated by N_2 second positive system ($C^3\,\Pi_u$–$B^3\sum_g$) and N_2

Fig. 3 The temperature of wheat seeds after plasma treatment



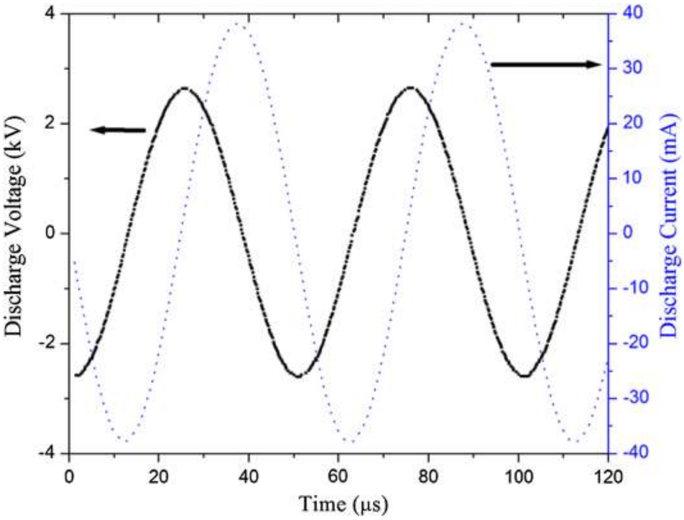


Fig. 4 The discharge voltage and discharge current at 14 l/min as a fixed flow rate

ion first negative system ($B^2\sum_u$–$X^2\sum_g^+$). The characteristic spectrum of the second positive system transitions is shown at 339.1 and 359.4 nm, while the nitrogen first negative system transitions is shown at 392.9 nm. The emission lines as can be seen in Fig. 6 were primarily dominated by the reactive species related oxygen (O I $^5S^0$-^5P) at 6.18.5 nm. On the other hand, another reactive atomic oxygen has been detected at 777.7 and 843.3 nm [49, 50]. Additionally, the reactive atomic nitrogen has been detected at 744.6, and

Fig. 5 Emission spectra from 200–500 nm observed inside the jet nozzle at 14 l/min as a fixed flow rate respectively

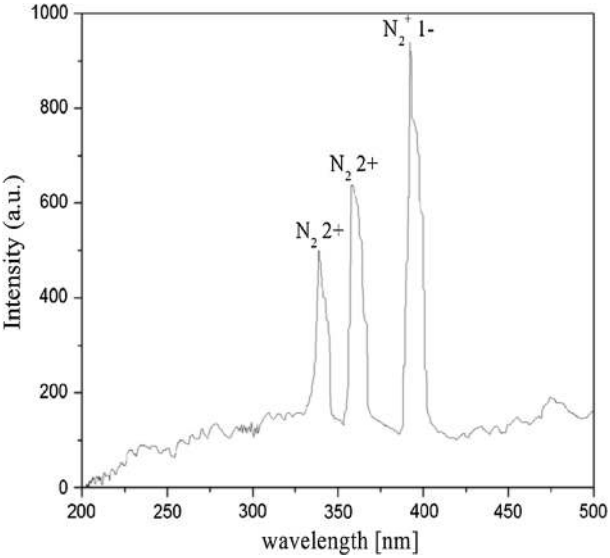
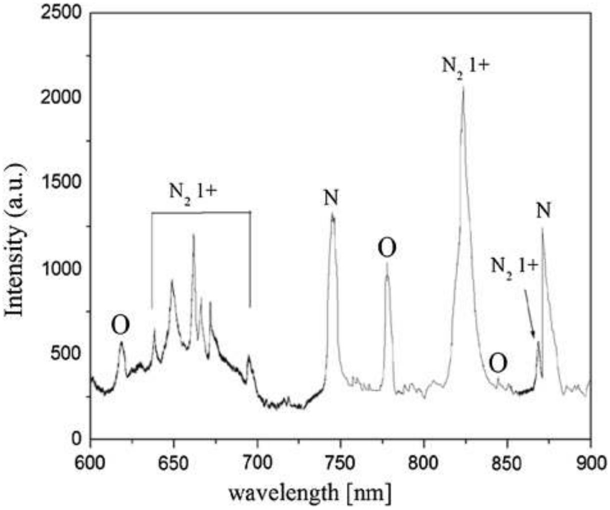


Fig. 6 Emission spectra from 600 to 900 nm observed inside the jet nozzle at 14 l/min as a fixed flow rate



870.8 nm [49, 51]. Another reactive spice has been detected is nitrogen first positive system ($B^3\Pi_g$–$A^3\sum_u^+$), these transitions are shown in the range of 639.1–695.02 nm and also detected in 824.6 and 867.3 nm.

Wettability Measurements

No doubt that, the seed coat of plants may hinder their ability to absorb water, which has a dramatic effect on the process of seed germination [52]. On the other hand, Yiran et al. (2017) observed that the morphology of the wheat seed coat was changed after plasma treatments; it has been led to enhancement of the water uptake and promotion of wheat seed germination was occurred [53]. From Table 1, it can be noticed that the mean wettability after 2 h was 19.12% for the controlled seeds but increased to 24.21%, 35.58%, 25.72%, 27.67% and 26.38% for the X_2, X_4, X_6, X_8 and X_{10} treatments respectively. On the other hand, it can observe that the wettability of X_2, X_4, X_6, X_8 and X_{10} treatments exhibit significant differences compared with the control seeds. However, there are no significant differences between the wettability of X_2, X_6, X_8 and X_{10} treatments. The results show that, the wettability of wheat seeds treated by cold atmospheric plasma jet increased with respect to untreated one. From these data, it can be noticed that, the nitrogen cold atmospheric plasma has a positive effect on the wettability of wheat seeds. However, there is a significant difference between treated and untreated wheat seeds after soaked in water for 4, 6, 8 and 10 h, While, there is no a significant difference between X_2, X_4, X_6, X_8 and X_{10} plasma treatments. From these results it can notice that, the treated seeds reached water- saturation faster untreated seeds because the wettability dynamic of treated seeds plasma decrease by increasing imbibition time. After soaked in water for 12 h there is no significant differences between all samples because the seeds were reached water- saturation in all variants [44, 52].Numerous studies have suggested that there might be some association between seed germination and water uptake, and the water uptake of seeds would benefit seed germination and seedling growth [35, 52]. In current work it can be found that nitrogen cold atmospheric plasma treatments enhanced the water uptake of wheat seed, and these results were consistent with the findings of Filatova et al. [54] and Bormashenko et al [29]. Filatova et al observed that the cold plasma treatment could improve the water uptake of some grains and legumes. Bormashenko et al. observed that the water uptake of oat was enhanced after RF plasma treatment. However, the wetting properties of seeds were altered due to oxidation of the seed surface by the plasma treatment, which would then improve water uptake and seed germination [29]. On the other hand, the wetting properties of oat and wheat seeds were improved after microwave plasma treatment, as well as their germination [32]. Moreover, the plasma treatment of seeds can be led to the change of the surface roughness and the chemical structure of grain and so that the shift of wetting characteristics has been occurred as well as affected its water uptake [55]. The enhancement of seeds ability to absorb water can be attributed to the improvement of the seeds hydrophilic by plasma treatment. However, the seeds ability to absorb water could be enhanced as well as the germination growth [22].

Table 1 Evolution of water uptake of the wheat seeds with CAPJ plasma treatment time, lowercase letters a–d in the same column represent significance analysis; the different letters mean significant difference among various treatments at $P < 0.05$ level

Plasma treatment (min)	Imbibition time					
	2 h	4 h	6 h	8 h	10 h	12 h
0 (X_0)	19.12 ± 0.63a	41.41 ± 0.74a	55.38 ± 1.23a	58.89 ± 0.60a	70.81 ± 0.50a	81.20 ± 0.60a
2 (X_2)	24.21 ± 1.09c	48.21 ± 1.02c	62.19 ± 1.06c	63.74 ± 0.59c	80.26 ± 0.95b	82.28 ± 0.92a
4 (X_4)	35.58 ± 1.37b	58.88 ± 0.55b	68.18 ± 1.01b	80.14 ± 1.09b	82.17 ± 0.79b	83.98 ± 0.23a
6 (X_6)	25.72 ± 0.74cd	48.27 ± 0.92c	62.01 ± 0.66c	72.88 ± 0.63c	81.22 ± 0.01b	83.25 ± 0.33a
8 (X_8)	27.67 ± 0.51d	48.31 ± 1.08c	64.74 ± 0.60c	75.03 ± 0.35c	80.64 ± 0.37b	81.08 ± 0.28a
10 (X_{10})	26.38 ± 0.16cd	48.55 ± 0.60c	64.25 ± 0.97c	74.13 ± 0.50c	80.22 ± 0.89b	80.93 ± 0.49a

Germination Characteristics

Seed dormancy is an intrinsic seed characteristic that allows the species to reproduce generatively to survive [56]. Non-thermal plasma is considered a novel stimulation treatment to break down dormancy [57]. Plasma treatment could generate UV radiation, radicals, and chemical reactions, which played a significant role in dormancy breaking. Non-thermal helium plasma might operate in dormancy breaking [58]. The germination results of untreated and treated wheat seeds after nitrogen cold atmospheric plasma jet were shown in Table 2. After one day of the plant, the mean germination potential was 21.4% in the untreated seeds, and it significantly increased to 91.3% in the X_4 treatment. Because the significant differences of the germination potential between treated and control seeds, it can be conclude that, the nitrogen cold atmospheric plasma enhancement the germination potential of wheat seeds. However, the X_6, X_8 and X_{10} treatments exhibit no significant difference between them. Also the nitrogen cold atmospheric plasma jet could improve the germination rate and germination percentage. In the case of germination rate, as can be seen in Fig. 7, 49.8% was obtained in untreated seeds, and it enhanced to 93.3% in X_4 treatment, and further increasing the treatment time did not improve the germination rate, because the difference among X_6, X_8 and X_{10} treatments was not significant. Moreover, the germination percentage increased from 67.9% in control seeds to 97.4% in the X_4 treatment. On the other hand, there was no significant difference among X_6, X_8 and X_{10} treatments. These results indicated that, the germination potential, germination rate and germination percentage for NCAPJ exhibited significant difference compared with untreated one. The highest mean germination potential, germination rate and germination percentage were obtained in the X_4 treatment. So that, we can conclude that, the enhancement of the wheat seeds germination depends on the cold atmospheric plasma dose, these results are consistent with the results obtained by Li et al. [33]. It is illustrated that the reactive oxygen and nitrogen species (RONS) are the essential signaling molecules organizing many growth processes in mammalian, microorganisms, and plants. The dose value of RONS can play an essential role in the control of growth and improvement of plants [59].

Seedling Growth at Different Plasma Dose

Table 3 displays the effects of NCAPJ treatment on the seedling growth of the wheat seeds. The mean root length was 1234.16, 1358.50, 1596.83 and 1375.40 mm for the control

Table 2 Effect of CAPJ plasma treatment time on the Germination characteristics for wheat seeds germination, lowercase letters a–f in the same column represent significance analysis; the different letters mean significant difference among various treatments at $P < 0.05$ level

Treatment time (min)	Germination potential %	Germination rate %	Germination percentage %
0 (X_0)	21.4 ± 1.0a	49.8 ± 1.3 a	67.9 ± 1.4a
2 (X_2)	75.4 ± 1.0b	75.7 ± 2.5 b	84.6 ± 1.6b
4 (X_4)	91.3 ± 1.1c	93.3 ± 1.8 c	97.4 ± 1.1d
6 (X_6)	61.6 ± 1.3d	74.3 ± 1.9 b	89.3 ± 0.9c
8 (X_8)	56.4 ± 1.1de	72.2 ± 2.5 b	80.2 ± 0.9b
10 (X_{10})	65.2 ± 2.0df	80.5 ± 1.6 bd	90.3 ± 1.1c

Fig. 7 The treated and untreated Wheat seeds after 4 days of cultivation; **a** untreated; **b** 2 min irradiated; **c** 4 min irradiated; **d** 6 min irradiated; **e** 8 min irradiated and **f** 10 min irradiated

seeds, X_2, X_4 and X_8 treatments, respectively, and the difference among these treatments was significant. However, the highest mean root length was obtained in the X_4 treatment. On the other hand, the significant difference between X_6 and X_{10} not exist. The mean shoot length increased to 473.30, 544.10, 504.70, 493.43 and 491.33 mm for the X_2, X_4, X_6, X_8 and X_{10} treatments, respectively, and these treatments presented significant differences compared with the control seeds (the mean shoot length was 357.03 mm). Moreover, a significant difference can be observed between T_4 treatment which represents the highest mean shoot length and the rest treatments. However, the mean shoot length of X_6, X_8 and X_{10} treatments do not exhibit significant difference. The mean fresh weight of the seedlings for the X_2, X_4, X_6 X_8 and X_{10} treatments were 1766.30, 1985.76, 1855.03,1795.33 and 1797.24 mg, respectively, which was 56.9, 76.5, 64.9, 59.5 and 59.7% higher than that of the untreated seeds, respectively. From these results, it can conclude that it can be noticed that, the mean fresh weight of treated seeds represents a significant difference with the control one, while the mean fresh weight of X_8 and X_{10} treatments do not exhibit significant difference. Moreover, the highest mean fresh weight was obtained in the case of the X_4 treatment. On the other hand, the mean dry weight was 567.34 mg in the untreated seeds and increased to 783.53, 875.72, 823.72, 765.72 and 764.30 mg for the X_2, X_4, X_6, X_8 and

Table 3 Seedling growth obtained from the control and plasmas treated wheat seeds lowercase letters a–e in the same column represent significance analysis; the different letters mean significant difference among various treatments at $P<0.05$ level

Treatment time (min)	Root length (mm)	Shoot length (mm)	Fresh weight (mg)	Dry weight (mg)
0 (X_0)	1234.16a	357.03 ± 2.59a	1125.10 ± 2.20a	567.34 ± 0.20a
2 (X_2)	1358.50b	473.30 ± 2.57b	1766.30 ± 2.07b	783.53 ± 0.01b
4 (X_4)	1596.83c	544.10 ± 2.35c	1985.76 ± 3.94c	875.72 ± 0.55c
6 (X_6)	1431.03d	504.70 ± 1.41e	1855.03 ± 1.90d	823.24 ± 0.03d
8 (X_8)	1375.40e	493.43 ± 0.90e	1795.33 ± 0.20e	765.72 ± 0.24e
10 (X_{10})	1457.56d	491.33 ± 1.15e	1797.24 ± 0.03e	764.30 ± 0.87e

X_{10} treatments, respectively. However, it can be observed that the mean fresh weight of treated seeds exhibited significant differences compared with the untreated seeds. However, the mean dry weight of X_8 and X_{10} treatment do not exhibit significant difference. The highest mean dry weight was obtained in the X_4 treatment which was 54.3% higher than that of the untreated seeds. Moreover, the effects of cold plasma treatment on seed germination and seedling growth of soybean have been studied by Li Let al. The authors improved that the higher and lower energy level doesn't enhancement the seedling growth of soybean, but the enhancement occurs moderate energy [33].The previous studies proved that the seedling growth of plants had been promoted by cold plasma treatment; the cold plasma treatment was enhanced the seedling growth of *Carthamus tinctorius* L. [60]; tomato [61]; wheat and oat [32], watermelon [34]. Moreover, [33, 62] have demonstrated that the seeds germination significantly increased by non-thermal plasma treatment.

Vigor Index

The seed vigor I and II were calculated as can be seen in Table 4 using equation No 5 and 6 respectively. From this table, the mean vigor index I was 1131.77 in the untreated seeds, and it increased to 1985.76 in the X_4 treatment. From these result it can notice that, the mean vigor index I between the treated seeds and the control seeds exhibit a significant difference. Also the difference among X_2, X_4 and X_6 treatments was significant, while there no significant differences between T_8 and T_{10} treatments. On the other hand, the X_4 treatment represents the higher vigor index II compared the rest treatments. However, the mean vigor index II was 382.84 in the untreated seeds, and it increased to 854 in the T_4 treatment. Moreover, the vigor index II of treated and

Table 4 Effect of CAPJ on vigor index, different lowercase letters mean significant difference among different treatments at $P<0.05$ level

Treatment time (min)	Vigor index I	Vigor index II
0 (X_0)	1131.77 ± 1.02a	382.84 ± 2.38a
2 (X_2)	1752.30 ± 2.50b	662.29 ± 1.12b
4 (X_4)	1985.76 ± 0.25c	854.00 ± 0.99c
6 (X_6)	1845.03 ± 1.23d	736.12 ± 0.81d
8 (X_8)	1785.33 ± 1.05e	615.13 ± 0.79e
10 (X_{10})	1787.24 ± 1.08e	691.82 ± 1.27f

untreated seeds exhibit a significant difference. Also the difference among treated seeds was significant. Lotfy concluded that the improvement of the vigor indexes of treated seeds can be attributed to the higher root and shoot length of treated seeds compared to untreated one [34]. From these results, it can concluded that the improvement of wheat vigor seeds which occurred by NCAPJ will be produced a high-quality yield in agricultural production. So that, NCAPJ treatment can be used to improve the vigor index in laboratory conditions. The last studies revealed that plasma-induced effects on the seed surface could produce to the permeation of RONS and UV inside seeds, which in all likelihood influence the physiological response, the seed germination, and the growth of plants [63, 64]. Therefore, from this work, it can be decided that the NCAPJ treatment of wheat seeds not only displayed a short term impact on the rate germination but also manifested a long term influence on the stem and the root growths.

Sprouts Weight

From Table 5 the mean weight of fresh sprout was 823.82, 1231.80, 1369.50 and 1342.46 mg for the control, X_2, X_4, and X_6 treatments, respectively, the difference among these treatments was significant, and the highest mean root length was obtained in the T_4 treatment. On the other hand, there is no a significant difference among $X_8$and X_{10} treatments. The same behavior was observed for the weight of dry spout. From these data, it could be observed that, the treated wheat seeds had a greater sprouts weight compared control one. From these results, it can be concluded that, the NCAPJ has a promise effect on germination improvement. Previous research showed that the dry mas of radish sprouts which treated with non-thermal plasma recorded higher value with concerning untreated one [65].

However, Krentsel et al. assumed that either of two processes or both accomplish the impact of the plasma treatment on seeds; that is, (1) a direct treatment on the seed coat and (2) an effect on the cells inside the seed. The direct treatment on the seed coat resulted in the etching influences by reactive spices and UV irradiation. On the other hand, the influence on the cells within the seed included the permeation of reactive spices. [66]. Sera et al. found that the contents of phenolic molecules in wheat and oat seeds modified after plasma treatment, and they ascribed these aspects to the permeation of plasma active species into the caryopses that consequently influenced metabolic processes [32]. Moreover, Zhang and Bjorn also suggested that UV radiation was a stressor that affected the content of phenolic compounds in organisms [63].

Table 5 Effect of CAPJ on the weight of fresh and dry sprout, different lowercase letters mean significant difference among different treatments at $P < 0.05$ level

Treatment time (min)	The weight of fresh sprout (mg)	The weight of dry sprout (mg)
0 (X_0)	823.82 ± 1.12a	324.75 ± 2.41a
2 (X_2)	1231.80 ± 0.56b	493.73 ± 2.19b
4 (X_4)	1369.50 ± 2.35c	593.63 ± 2.70c
6 (X_6)	1342.46 ± 0.12d	525.26 ± 3.82d
8 (X_8)	1293.02 ± 0.68e	562.43 ± 1.62e
10 (X_{10})	1292.19 ± 0.56e	567.53 ± 4.16e

Conclusions

A nitrogen cold atmospheric plasma jet device has been designed, constructed and operated in our laboratory. Spectroscopic measurements were performed to identify the constituent particles and estimate the rotational temperature of a nitrogen atmospheric plasma jet with a long plume. Spectroscopic measurements revealed that the particles with relatively high energy excited states exist inside the jet nozzle. The nitrogen cold atmospheric plasma jet device has been used to study the effect of non-thermal plasma on wheat seeds germination, seedling growth and vigor index. The treatment with our device did not cause any damage to the wheat seeds. The enhancement of wettability of the treated seeds can be attributed to the substantial effect of UV radiation which considered as the main component of cold atmospheric plasma. Cold atmospheric plasma plays an important role in penetrating some active compounds during the cell membrane of the wheat seeds, which leads to accelerated germination process. Under the effect of plasma generated by nitrogen cold atmospheric plasma jet not only the germination rate was stimulated, but also the vigor index of wheat. Nitrogen cold atmospheric plasma treatment leads to the dramatic effect on the seedling growth of wheat seeds. The results showed that the exposure of wheat seeds to plasma for 4 min considered the optimum condition of nitrogen cold atmospheric plasma jet treatment.

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