The effect of low-pressure dielectric barrier discharge (LPDBD) plasma in boosting germination, growth, and nutritional properties in wheat

Mahedi Hasan
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Md. Sohanur Rahman Sohan
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Salek Ahmed Sajib
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Md. Forhad Hossain
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Masum Miah
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Md. Mahmudul Hasan Maruf
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Khandaker Md. Khalid-Bin-Ferdaus
Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Ahmad Humayun Kabir
Molecular Plant Physiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205

Mamunur Rashid Talukder
Plasma Science and Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi, Rajshahi-6205

Md. Mamunur Rashid
Plasma Science and Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi, Rajshahi-6205

Md. Moinuddin

Oils, Fats and Waxes Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi Laboratories, Rajshahi-6205

Mona M. Elseehy

Department of Genetics, Faculty of Agriculture, University of Alexandria, Alexandria 21545

Ahmed M. El-Shehawi

Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944

Md. Abu Reza (reza.gen@ru.ac.bd)

Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205

Research Article

Keywords: plasma agriculture, low-pressure dielectric barrier discharge (LPDBD) plasma, wheat

Posted Date: June 8th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-575443/v1

Version of Record: A version of this preprint was published at Plasma Chemistry and Plasma Processing on November 18th, 2021. See the published version at https://doi.org/10.1007/s11090-021-10217-z.

The effect of low-pressure dielectric barrier discharge (LPDBD) plasma in boosting germination, growth, and nutritional properties in wheat

Mahedi Hasan[1], Md. Sohanur Rahman Sohan[1], Salek Ahmed Sajib[1], Md. Forhad Hossain[1], Masum Miah[1], Md. Mahmudul Hasan Maruf[1], Khandaker Md. Khalid-Bin-Ferdaus[1], Ahmad Humayun Kabir[2], Mamunur Rashid Talukder[3], Md. Mamunur Rashid[3], Md. Moinuddin[4], Mona M. Elseehy[5], Ahmed M. El-Shehawi[6], Md. Abu Reza[1*]

[1]Molecular Biology and Protein Science Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh

[2]Molecular Plant Physiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh

[3]Plasma Science and Technology Laboratory, Department of Electrical and Electronic Engineering, University of Rajshahi, Rajshahi-6205, Bangladesh

[4]Oils, Fats and Waxes Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi Laboratories, Rajshahi-6205, Bangladesh

[5]Department of Genetics, Faculty of Agriculture, University of Alexandria, Alexandria 21545, Egypt

[6]Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

∗Corresponding author: Professor Md. Abu Reza
E-mail address: reza.gen@ru.ac.bd

Abstract:

Plasma agriculture is an emerging technology, although the application of non-thermal plasma in wheat productivity is still in its early stage. This study deciphers the effect and mechanistic basis of non-thermal air-generated LPDBD (low-pressure dielectric barrier discharge) plasma in boosting germination, growth and nutritional properties in wheat. Seeds treated with LPDBD plasma exhibited cracked periphery and 6 min treatment showed a 22.11% increase in the germination rate compared to non-treated controls. At the cellular level, the concentration of H_2O_2 in leaves significantly increased (3.56 μM g^{-1}FW) due to LPDBD plasma treatment, may act as a stimulating agent to trigger the physiological functions in wheat plants. In addition, plants sprouted from air-treated seeds exhibited a marked elevation in CAT and SOD activity accompanied by the increased expression of *TaCAT* and *TaSOD* genes in roots of wheat. Interestingly, the grain yield of wheat increased by 27.06% in response to plasma treatment compared to control. Further, grains harvested from plasma-treated plants showed a substantial elevation in iron and fat content as well as decreased moisture content that may contribute to the increased shelf life. The study will open up a new avenue for practical application of plasma in agriculture.

Introduction

For several years a huge number of people around the globe can not meet their daily food needs without charitable assistance, primarily driven by two factors: persistent instability in food price and adverse climatic events [1]. Food prices rushed in 2008, sinking millions into hunger and triggering riots in developing countries [2]. The United Nations Food and Agriculture Organization (FAO) indicated that in 2010, the entire global production of grain was accounted for about 2.216 billion tons against a consumption rate of 2.254 billion tons. This resulted in the starvation of nine million people, consequentially an elevation in demand of food is occurring on a par with the rapid population expansion globally [3]. An analysis of the Food and Agriculture Organization (FAO) predicted a remarkable extension in the world population by 2050. Based on this forecast, FAO specified that agronomic yield should be extended in the upcoming year by 60% globally [4,5]. As the cultivatable land is steadily decreasing, food safety can only be ensured by an exponential increment of crop per unit production. Fertilization [6] and Irrigation [7] is accessible technology to progress production, although sometimes these technologies are limited by the farmers' economy [8] and they do not ensure an uninterrupted ecological equilibrium [9,10]. Molecular breeding [11] and genetic engineering [12] are more modern approaches to increase gross production, however, they are time consuming. The application of cold plasma technology is new in agriculture as a viable, cost effective, eco-friendly, and non-hazardous measure by creating a new way for developing grain yield. It could act as a stimulant for the vigor of seed keeping the risk of genetic mutation at minimum, as it is based on non-ionizing low-level radiation. In many countries, extensive research has been carried out on the agricultural application of plasma technology. It was found the growth and yield of lettuce [13], cucumber [14], tomato [2], soybean [15], rice [16], spinach [17], peanut [18], eggplant [19], maize [20], rape [21,22], oat [23], radish [24] black gram [25,26], and *Andrographis paniculata* [27,28] were improved after treated with plasma. Although wheat (*Triticum aestivum* L.) is a staple crop in many countries, it's yield is being steadily reduced because of several environmental factors (changes in global temperature, humidity, and drought, etc.) and manmade adversities [15,29,30] leading to malnutrition which is a current crisis worldwide. Hydrogen peroxide (H_2O_2) is an important REDOX (reduction-oxidation reaction) metabolite and causes oxidative damage to biomolecules at high concentrations, which culminates in cell death. However, H_2O_2 acts as a signalling molecule at concentrations in the nanomolar range and resembles phytohormones in certain ways. A number of excited, reactive oxygen and nitrogen (ROS, RNS) molecules, free radicals, ions, photons are created by plasma treatment, and the high temperature of electrons helps in generating these reactive species. Plants activate their defense mechanisms such as enzymatic and non-enzymatic antioxidants when encounter stress such as ROS and RNS (O_3, OH-, H_2O_2, NO, NO_2, O_2, O) [31,32]. The antioxidant enzyme's coordinated action scavenges the activity of ROS and RNS [33,34]. The interaction of plasma with cell metabolites could enhance the activities of seedling germinating enzymes [35] and accelerate the decomposition of the inner nutrients of the seed, which could lead to increased use of the seed reserve and growth of the seedling. As research on the effect of dielectric barrier discharge (DBD) or non-thermal air plasma is new in wheat, the fundamental purpose of the current study is to inquire the following questions a) studying the effect of air plasma on seed germination that reduces the total number of seeds required for cultivation; b) investigating the mechanistic basis of

plasma-induced improvement of wheat, and c) studying the effect of plasma on physicochemical and nutritional composition in wheat.

Results

Wheat seed germination and Plant growth characteristics

The germination of wheat seeds was prompted by LPDBD plasma treatment. The mean germination, root length, shoot length, fresh weight, and dry weight were shown in Supplementary table S1. The mean germination rate was significantly improved to 89.17%, 96.67%, 90.83%, and 88.33%% for the 3 min, 6 min, 9 min, and 12 min of plasma treatments respectively compared to the control. However, the highest germination rate achieved by 6 minutes treated seeds showed significant difference with 1 min, 3 min, 9 min, and 12 min treated seeds. Thus, cold plasma treatment increased the germination rate by 4.21%, 12.63%, 22.11%, 14.74%, and 11.58% in 1 min, 3 min, 6 min, 9 min and 12 min treatment, respectively, compared to the control. All the treatments improved plant growth characteristics. The mean root length increased to 19.01cm, 19.06 cm, and 19.48 cm for the 1 min, 3 min, and 12 min treatments, respectively, and these treatments presented no significant differences compared to the control (the root length 15.98 cm). The mean root length was 19.94 cm, 20.16 cm for the 6 min, and 9 min treatment, which exhibited a significant difference compared to the control. The shoot length was 65.39 cm, 70.67 cm, 76.06 cm, 76.66 cm, 80.07 cm, and 74.51 cm in control, 1 min, 3 min, 6 min, 9 min, and 12 min treatments, respectively. Among them 3 min, 6 min, and 9 min treatments presented significant difference compared to the control. The highest root length and shoot length reached 80.07 cm, 20.16 cm in 9 minutes treatment, which was significantly higher compared to the controls by 22.45% and 26.15% respectively. The dry weight of plants from the 1 minute, 3 minutes, 6 minutes, 9 minutes, and 12 minutes treated seeds were increased by 3.92%, 28.23%, 98.04%, 76.86%, and 18.43% respectively, compared to control in which 6 minutes and 9 minutes treatment were statistically significant compared to the control. However, a slight increase was found in fresh weight, but that was not statistically significant. The average chlorophyll contents, numbers of tiller, stem diameter, plant height, panicle length, panicle diameter were shown in Supplementary table S2. The uppermost mean chlorophyll content was acquired in the 6 min treatment, which represented a significant difference compared to the control, and increased by 27.10% compared to the control. The uppermost mean number of tillers was 7.78 for 6 min treatments, which significantly differed from the control, 1 min, 3 min, and 12 min treatments. While the average number of the tiller for 9 min treatments was 6.56, that is significantly different from the control, but exhibited no significant difference with 1 min, 3 min, 6 min, and 12 min treatments. The mean stem diameter was improved to 4.93 mm and 4.22 mm for 6 min and 9 min treatments, that was exhibited significant difference compared to the control (the mean stem diameter 3.46 mm), in which the uppermost stem diameter obtained in 6 min treatments exhibited significant difference from all others treatment. The uppermost mean plant height was 94.88 cm for 6 min treatment that exhibited significant difference compared to the control (73.31 cm), 1 min (80.84 cm), and 3 min (86.1 cm) treatments. The average height of 9 min and 12 min treatments was 90.44 cm and 88.45 cm, and they were exhibited a significant difference

compared to the control but showed no significant difference with 1 min and 3 min treatments. The average panicle length was improved to 19.22 cm and 18.63 cm for 9 min and 12 min treatment, which exhibited significant difference, compared to control (15.73 cm) but showed no significant difference compared to the 6 min treatment and between them. However, the uppermost mean panicle length was 20.72 cm for 6 min treatment, which was significant compared to control, 1 min, and 3 min treatments. The mean panicle length was increased by 11.79%, 21.26%, 31.7%, 22.18%, and 18.43% for 1 min, 3 min, 6 min, 9 min, and 12 min treatment correspondingly compared to the control. The average panicle diameter was 14.98 mm, and 13.62 mm for 6 min and 9 min treatment, which was significantly different compared to the control and 1 min (9.91 mm), and significantly higher than the control (8.06 mm) by 85.93% and 69.10%, but exhibited no significant difference compared to the 3 min and 12 min treatments and between them. The mean panicle diameter for 3 min (11.89mm) and 12 min (12.24 mm) exhibited significant differences compared to the control. The average panicle diameter for 1 min treatment exhibited no significant difference than control.

The uppermost mean number of grains per panicle was 287, which is significantly higher compared to the control (210). Thus, 6 min plasma treatment caused 36.67% increase in grain number per panicle. Seeds treated for 1 min, 3 min 9 min, and 12 min also showed marked improvement in terms of grains per panicle which were 247.67, 270.33, 234.33 and 221.67 respectively compared to the (Table 1). The average number of grain per panicle was 270.33 for 3 min treatments, which presented a significant difference compared to the control but exhibited no significant difference compared to the 1 min, 6 min, and 9 min treatments (Table 1). The average number of grains per panicle was improved by 17.94%, 28.73%, 11.59%, and 0.79% for 1 min, 3 min, 9 min, and 12 min treatments, respectively, compared to the to the control. The mean thousand grains weight was 39 g for 6 min treatment, which presented a significant difference compared to the control (32 g) and increased by 22.92% compared to control (Table 1). The average thousand grains weight improved to 35 g, 36 g, 37.67 g, and 36.67 g, for 1 min, 3 min, 9 min, and 12 min treatments respectively, presented no significant difference compared to the control and increased by 9.38 %, 12.5%, 17.71%, and 14.58% respectively compared to control (Table 1). The uppermost mean yield m^{-2} was 272.3 g for 6 min treatment, which was significantly higher than the control (214.33 g) by 27.06% (Table 1). The average yield per meter square was 225.33 g, 228.33 g, 245.67 g, and 238.67 g for 1 min, 3 min, 9 min, and 12 min treatments, respectively, which were higher than the control by 5.13%, 6.53%, 14.62%, and 11.35% respectively, but they were not statistically significant (Table 1).

Scanning Electron Microscope (SEM) Analysis of wheat Seeds surface

The wheat seed surface exhibited a rectangular shape of sub-domain with a distinct edge before the air plasma treatment (Fig. 1(a)). In contrast, the rectangular shape sub-domain was completely disappeared after the air plasma treatment (Fig. 1(b)). Besides, cracks were noticed on the seed coat after the plasma treatment (Fig. 1(b) arrowed).

Estimation of soluble protein, soluble sugar, H_2O_2 activity, and NO activity

The highest mean hydrogen peroxide (H_2O_2) activity in leaves and roots were 3.56 µmol g^{-1} FW and 4.55 µmol g-1 FW respectively for 6 min treatment in which leaves H_2O_2 activity

were significantly higher compared to the control (1.25 µmol g^{-1} FW) and 1 min (1.81µmol g^{-1} FW) treatment; while root H_2O_2 activity exhibited no significant difference compared to the control (3.12 µmol g^{-1} FW) and other treatments (Fig. 2(a),Fig.2(b)). The average H_2O_2 activity in leaves were 2.45 µmol g^{-1} FW, 2.08 µmol g^{-1} FW, and 2.66 µmol g^{-1} FW for 3 min, 9 min, and 12 min treatments respectively, which presented significant difference compared to the control and 1 min treatments (Fig.2 (a)). The upper most mean nitric oxide (NO) activity in leaves and roots was 6.68 µmol g^{-1} FW and 5.83 µmol g^{-1} FW for 6 min treatment respectively, in which leaves NO activity presented significant difference compared to the control (2.95 µmol g-1 FW) and 1 min (3.67 µmol g^{-1} FW), 3 min (3.75 µmol g^{-1} FW), and 12 min (4.71 µmol g^{-1} FW) treatments while roots NO activity presented no significant difference compared to the control (3.77 µmol g^{-1} FW) and other treatments (Fig. 2 (c),Fig. 2 (d)). The uppermost mean soluble protein in leaves was 36.15 mg g^{-1} FW, which presented a significant difference compared to the control (23.92 mg g^{-1} FW), and 1 min (24.74 mg g^{-1} FW) treatments (Fig.2 (e)). The average soluble protein in leaves for 3 min, 9 min, and 12 min treatments presented no significant difference among them and other treatment (Fig. 2 (e)). The uppermost mean soluble protein in roots was 47.59 mg g^{-1} FW for 6 min treatment, which showed a significant difference compared to the control (21.32 mg g^{-1} FW), 1 min (24.03 mg g^{-1} FW), 3 min (33.82 mg g^{-1} FW), and 12 min (34.28 mg g^{-1} FW) treatments (Fig. 2 (f)). Average roots soluble protein activity in 9 min treatment presented substantial difference compared to the control and 1 min treatments, while roots soluble protein activity in 12 min treatment presented a significant difference only compared to the control (Fig. 2 (f)). The uppermost mean soluble sugar in leaves and roots were 3.04 mg g^{-1}FW, and 3.6 mg g^{-1} FW correspondingly, which did not present any significant difference compared to the control (Fig. 2(g), Fig. 2(h)).

Antioxidant enzymes

The Catalase (CAT) activity in the leaves and roots is improved gradually up to 6 min and thereafter it is decreased (Fig. 3 (a), Fig. 3 (b)). The maximum mean catalase (CAT) activity in leaves and roots was 1.03 nmol min^{-1}(mg $protein^{-1}$) and 0.32 nmol min^{-1}(mg $protein^{-1}$) for 6 min treatment respectively, in which leaves CAT activity presented significant difference compared to the control and increased by 222.02% compared to the control (Fig. 3 (a). In contrast, maximum mean CAT activity in roots exhibited no significant difference compared to the control (Fig. 3 (b) and increased by 77.77% compared to the control (Fig. 3 (b). However, no other treatments CAT activity in leaves and roots presented significant difference compared to the control (Fig. 3 (a), (Fig. 3 (b)). The expanding tendencies of SOD activity were witnessed together in leaves and roots as displayed in (Fig. 3 (c), (Fig. 3 (d)) correspondingly, where the maximum SOD concentrations were 7.71 nmol min^{-1}(mg $protein^{-1}$) and 3.76 nmol min^{-1}(mg $protein^{-1}$) respectively, achieved by the plantlets produced from the seeds treated for 6 min, in which leaves SOD activity presented significant difference compared to the control and increased by 162.44% compared to control (Fig. 3 (c)). In comparison, roots SOD activity showed no significant difference compared to the control and other treatments and increased by 27.81% compared to control (Fig. 3 (d)). In other treatments, SOD activity in leaves and roots presented no significant difference compared to control and among them (Fig. 3 (c), (Fig. 3 (d)). The expanding tendencies of

APX and GR activity were witnessed together in leaves and roots as displayed in (Fig. 3 (e), (Fig. 3 (f)) and (Fig. 3 (g), (Fig. 3 (h)) correspondingly, where the maximum APX concentrations were 0.80 nmol min^{-1}(mg $protein^{-1}$), and 0.86 nmol min^{-1}(mg $protein^{-1}$) and GR concentrations were 0.26 nmol min^{-1}(mg $protein^{-1}$) and 0.54 nmol min^{-1}(mg $protein^{-1}$) respectively, achieved by the plantlets produced from the seeds treated for 6 min. However, none of these treatments statistically significant compared to control.

Gene expression correlated with antioxidant activities

Our study exhibited noteworthy up regulation of *TaCAT*and *TaSOD* expression in roots of wheat seedlings grown from 6 min air plasma treated seeds related to controls (Fig. 4).

Evaluation of food values

The lowest mean moisture content was 9% for 6 min treatment, which presented significantly decreased compared to the control and other treatments. In contrast, other treatments demonstrated no significant decrease compared to the control and among them (Table 2). The uppermost mean fat content was 1.73% for 6 min treatment, which presented a significant difference compared to the control, and increased by 70.62% compared to control, while other treatments also presented significant difference compared to the control and exhibited no significant difference among them (Table 2). The mean fat content was increased by 43.79%, 54.07%, 53.78%, and 40.72% for 1 min, 3 min, 9 min, and 12 min treatments respectively, compared to control. There were no significant changes found in mean crude fiber, ash, and protein content in any treatments compared to control and among them. The highest mean crude fiber, ash, and protein content were 0.93%, 2.65%, and 11.85 for 6 min, 1 min, and 9 min treatments respectively (Table 2).

Grain and leaves trace elements

Grain Fe is improved gradually up to 6 min(Fig. 5 (a)).The uppermost mean grain Fe was 30.75 mg/kg for 6 min treatments, which showed a significant difference compared to the control and increased by 36.89% compared to control, while other treatments presented no significant difference compared to the control and among them (Fig. 5 (a)). The uppermost mean leaves Fe was 206.19 mg/kg for 6 min treatments, which showed a significant difference compared to the control and increased by 21.99% compared to control, while other treatments showed no significant difference compared to the control and among them (Fig. 5 (b))**.** A slight enhancement is noticed in Zn and Mn concentration in grains and leaves as compared to control, while none of these treatments presented a significant difference compared to the control and among them (Fig. 5 (c), (Fig. 5 (d);(Fig.5 (e);(Fig. 5 (f))).

Discussion

Many nations of the universe are in a threat of food safety with growing need due to enhanced inhabitants and decrease in implanted area and adverse climatic changes. The production of wheat along with other crops is declining due to the alteration of weather [29,30] and severe reductions in implanted space globally. The current study revealed the effect of non-thermal plasma (generated from air) on the improvement of quantity and quality of wheat production. The 6 min Air plasma treatment increased the germination rate up to 22.11% compared to the control, which will reduce the number of seeds required for cultivation (Supplementary table S1). Few other studies also have conveyed that non-thermal plasma significantly improved seed germination [15,36–38]. Our result was coherent with those

conveyed previously [28], a suitable air DBD plasma treatment could improve wheat seed germination in laboratory conditions. In the current experimental setup, LPDBD plasma also improved seedling growth and dry matter accumulation such as the shoot length, root length; tiller number, stem diameter, plant height, fresh weight, and dry weight which were increased by 22.45%, 26.15%, 105.88%, 42.77%, 25.98%, 19.09%, and 98.04% respectively compared to the control for 9 min, 9 min, 6 min, 6 min, 6 min, 6min, and 6 min treatment correspondingly (Supplementary table S1) and (Supplementary table S2). Similar studies in some other plants have revealed that non-thermal plasma treatments stimulate plantlet development [2,37,39]. Our experiment showed that chlorophyll content, panicle length, panicle diameter were also increased by 0.42%-21.1%, 11.79%-31.71%, 23.03%-85.93% respectively, compared to control due to the different duration of plasma treatments (Supplementary table S2). Higher chlorophyll content provides the plant added advantage for better photosynthesis which ultimately contributes in increasing grains size, panicle length, and diameter. Air plasma treatment increased grains per panicle, thousand grains weight, and yield m^{-2} by 0.79%-36.67%, 9.38%-22.92%, and 5.13%-27.06% respectively compared to control due to different duration of plasma treatments (Table 1) and the highest achievement was shown by 6 min of air plasma treatment (Table 1). Atmospheric pressure air plasma treatment causes increased gas temperature (~40 ° C) inside the discharge vessel which release energy as heat by the plasma species such as ROS and RNS. This results the seed coat starch and protein to interact with the generated oxygen and nitrogen related species, viz. reactive O, OH, NO_2, N_2O, NO, CO_2, HNO_2, HNO_3 [40], results in more permeable seed surface compared to the control (Fig. 1). Several scholars have suggested that air plasma prompted reactions on the seed surface might consequence in a greater invasion of ROS, RNS, and UV radiation, which in turn assists in various physiological responses [38,41]. These chemical reactions stimulate biological stimulation, which promotes seed germination [42]. It is found that air plasma treatment changes seed coat structure and roughness (Fig. 1 (b)), which enhances water uptake ability results in greater germination and seedling growth [15]. Soluble protein and soluble sugar activity have important tasks in growth and adaptive responses [43]. Jiang et al. (2018) reported that non-thermal plasma treatment significantly improved the absorption of nitrogen (12.7%), which in turn enhancing protein content [44]. Soluble sugar is thought [45] to deliver an adaptive response to drought, low temperature, pathogen challenge, anoxic injury, and excess excitation energy. The activity of soluble sugar was improved in seedling (Fig. 2. g, h) which might aid for the endurance of plantlets from anoxic injury. In the current studies, after LPDBD plasma treatment, the soluble protein content activity of wheat seedlings was enhanced compared to those of the controls (Fig. 2 (e), (Fig. 2 (f)). In corn seedlings, similar findings were reported by Wu et al. [46].

The germination rate of seed is found to increase with treatment duration and reaches the highest level after that it is reduced (Supplementary table S1). This phenomenon can be described as nitrogen (N) complex is a reserve compound in many seeds, which play a significant role in faster and increased germination [47] of wheat seeds. We also found the highest nitrogen activity after that it is reduced (Fig. 2 (c), (Fig. 2 (d)). This phenomenon is consistent with the seed germination rate. Increased nitrogen content not only plays a significant role in enhanced germination [48] but also improve plantlet growth with the maximum extension of leaves [47]. Moreover, enough N content [49] and increased chlorophyll

content in the leaves. The outcomes of this study concerning the improved root and shoot lengths because of enhanced contents of nitrogen in the seed as reported earlier [49]. Thus, the LPDBD air plasma treatments can enhance the N content in the seeds that function as reserved nitrogen. Further, the reserved N content of the seed is distributed [50] among proteins and amino acids. Subsequently, it is thought that the N enriched seeds can generate enough amino acids and proteins through the metabolization of the N complex and provide requisite nutrients that can improve plantlet development and chlorophyll concentration in the leaves. Hydrogen peroxide (H_2O_2) concentration is increased in the seedlings due to plasma treatment (Fig. 2 a, b). Although H_2O_2 is an initiate of a stress factor in plants but the controlled [45] amount of H_2O_2 function as the signal transduction for soluble sugar synthesis, therefore slightly increased up the soluble sugar content (Fig. 2 g, h) in the seedlings. CAT and APX are mainly the scavengers of H_2O_2 in which CAT exhibited significant changes in leaves subjected to air plasma (Fig.3 (a)). The leaves exposed to more oxidative stress rather than the roots due to air plasmas treatment or environmental stress. When cells are stressed for energy and are rapidly producing H_2O_2 via catabolic processes, H_2O_2 is not threatening or toxic to plants and H_2O_2 is degraded by CAT enzymes in an energy-efficient way resulting from the expression of the gene *TaCAT* (Fig. 4). While APX activity is slightly increased in roots and shoots (Fig. 3 (e), (Fig. 3 (f)), but it is statistically not significant. These two antioxidant enzymes work redoxly to remove H_2O_2, while little APX activity would be led to the slight accumulation of H_2O_2 in seedlings, which enable the plants to tolerate stress [25]. SOD is one of the major antioxidant enzymes elevated against oxidative stress produced by reactive oxygen. SOD is the only enzyme which acts on superoxide radical dismutase to hydrogen peroxide and oxygen. The SOD activity is found statistically significant in leaves compared to the control (Fig. 3 (c) which was further supported by the *TaSOD* expression (Fig. 4). Improved activities of SOD suggest enhanced production of superoxide anion in seedling which indicates that the plant's defense mechanism becomes enriched. This result is reliable with the findings of [51]. Glutathione reductase (GR) is an enzyme that catalyzes the reduction of glutathione disulfide (GSSG) to glutathione (GSH). GR activity is slightly improved in roots while in leaves it is almost similar compared to the control (Fig. 3 (g), (Fig. 3 (h)). Moisture contents were determined to measure the level of water in wheat grains, which is an important factor in terms of productivity [52]. Moisture content range between 9.0%-11.45% for different duration of treatment, among them moisture content of grain obtained from the plant from 6 min treated seed showed significant difference compare to others (Table 2). The moisture content of different wheat varieties is estimated to range from 9.90 to 12.48 percent [53]. Thus, plasma treatments slightly reduce moisture content; protect the grain from the microorganism. Plasma treatment significantly increased fat content compared to the control. The mean fat content range between 1.02% -1.73% (Table 2). This result is supported by the result of [54]. The highest fat content increased by 70.62% compared to control. The mean crude fiber, and ash content range from 0.81%-0.93%, and 2.29%-2.57% respectively (Table 2). This result is supported by the result of [55] and [56], respectively. The mean protein content range from 11.56-11.85 mg/g (Table 2). There are no significant changes found in mean crude fiber, ash, and protein content of wheat grain. Thus, plasma treatment only improves fat content and reduces moisture content in wheat grain, though the reason is indistinct. The higher-yield wheat genotypes have been reported to be associated

with lower Fe, Mn, Zn, and Cu concentrations [57]. Fe and Zn the metals which are most often considered deficient in plants, and consequently in the human diet. In both leaves and grains of wheat plants subjected to air plasma treatment in seeds, we observed a substantial increase in Fe. Iron uptake depends on the ability of the plant to reduce Fe^{3+} to Fe^{2+} through the electrons at the surface of the cell [58]. While Zn and Mn concentration slightly increased in leaves and grains but it is statistically not significant compared control (Fig. 5 (c), (Fig. 5 (d) and (Fig. 5 (e), (Fig. 5 (f)).

Materials and methods

Seed collection

Wheat seeds (Bari 21) were collected from local market. Seeds were sensitively selected so that all the seeds are similar in size and have no scratches.

LPDBD plasma generation and seed treatment

Two copper electrodes (diameter 9 mm, thickness 0.5 mm) were placed axially at the two ends of the test tube (diameter 12 mm, length 50 mm) as shown in fig. 6. The powered electrode was covered with a pyrex glass disk used as dielectric layer. The gaps between the electrodes were kept at 40 mm. Wheat seeds were kept in the space between two electrodes. A vacuum pump decreased the pressure inside the chamber and the pressure was maintained at~$10\ torr$. After that, a high voltage (5–10 kV, 3–8 kHz) was provided to the electrodes for plasma generation and treating the seeds. During seed treatment, air was supplied to the chamber, and flow was controlled by a gas flow controller Yamato, KIT and was maintained at $1\ l/m$. The wavelength of the discharge, voltage (HVP-08), and current (CP-07C) were recorded in a digital oscilloscope (GDS-1000B). The emitted spectra produced in the plasma were recorded with spectrometers (USB2000 + XR1, slit size 25μm, grating 800 lines/mm, optical resolution (107nm) in the wavelength range from 200 to 1100 nm for the identification of species. High resolution dual-channel spectrometer (AVASpec-2018, slit size 10μm, grating 2400 lines/mm, optical resolution 0.07 nm) was used in the range from 200 to 500 mm for the estimation of plasma parameters. Seeds were treated for 1 min, 3 min, 6 min, 9 min, and 12 min by air plasma.

Scanning Electron Microscopy (SEM)

Seeds were dried in an oven at 30ºC overnight to remove moisture. After that, plasma treatment was performed and immediately carried out the scanning electron microscopy (SEM) by FEI S50 scanning electron microscope (FEI Technologies Inc., Oregon, United States) using ZEISS software at 10 μm scales, and changes found in the seed coat were compared to the non-treated controls**.**

In Vitro seed germination assay

Forty (40) control (non-treated) and treated seeds were immersed for 5-h in deionized water and subsequently kept in petri dishes for germinating. Two layers of wet filter paper were placed into sterile petri dishes, and 40 wheat seeds were placed in each Petri dish. The petri dishes were kept in a germination chamber at 25°C, 12 h light/ 12 h dark photoperiod (flux intensity 120μmol m^{-2} s^{-1}), and 60% air humidity. Extra deionized H_2O was sprinkled daily to sustain an adequate amount of moisture for germination. After 4 days, the germinated seeds were separated and the germination percentage (GR, %) was calculated by the formula

mention bellow (the radicle projection at one mm was considered as the criterion for germination) :

$$GR = (SG/ ST) \times 100\ \%$$

Where,

GR=germination rate

SG=number of germinated seeds

ST=total number of seeds.

Field preparation and seeds sowing

In this study, the randomized complete block design (RCBD) method was followed for field preparation. Plot length was 4 m^2, line-line spacing (72cm) and every condition had three replications and the total number of the plot was 18 with control. The seeds were sowed randomly in the field after plasma treatment.

Growth parameter

Germinated plantlets were collected from the field for growth parameter analysis, such as roots length, shoots length, number of tiller, fresh weight, and dry weight after 30 days of sowing. Three replications from each plot were taken and mean value was considered for each trait. Sufficient amounts of roots and leaves were stored at minus 20°C for further analysis. After 40 days of sowing, plant height, stem diameter and after 90 days length of panicle, the diameter of panicle, and plant height with panicle were determined using tailoring tape scale and vernier caliper whichever applicable. Chlorophyll content was also measured at LEAF CHL STD Chlorophyll meter (FT GREEN, USA).

Catalase (CAT), Superoxide dismutase (SOD), Ascorbate peroxidase (APX), and Glutathione reductase (GR) activities in roots and leaves

Enzymes activities were evaluated as per earlier report with minor changes [59]. In brief, tissues were crushed in phosphate buffer (100mM, ph=7.0) using mortar and pestle and centrifuged for 8 min (10000 rpm) before separating the aliquot in a fresh tube. The activity of antioxidant enzymes (CAT, SOD, GR, and APX) was evaluated using spectrophotometric method, as illustrated earlier [59].

Relative gene expression investigation

The relative expression of *TaSOD,* and *TaCAT* transcripts was carried out in roots by qPCR following the previous reports [48,60]. 50 mg roots were crushed with mortar and pestle into liquid nitrogen. Complete RNA was isolated using the SV Total RNA Isolation kit (Cat. no. Z3100, Promega Corporation, USA) using the manufacturer's protocol. I, the Integrity of RNA samples was examined on formamide gel electrophoresis. RNA was quantified and purity of RNA was checked using NanoDrop2000 (Thermo Scientific, USA). 1 µg of total RNA was reverse transcribed into first-strand cDNA using GoScriptTM Reverse Transcription kit (Cat no. A5001, Promega Corporation, USA). To eradicate the risk of any RNA carryover cDNA was treated with RNaseH. Finally, real-time PCR analysis was performed in an EcoTM real-time PCR (Illumina, USA) device operated by Eco Software v4.0.7.0. Sequences of gene specific primers used in real time PCR are given in Supplementary table S3. As an internal control, expression analysis was standardized with β-

Actin. The PCR software in real time was used as follows: 10 min at 95 °C, 40 cycles of 10 s at 95 °C, 30 s at 55 °C and 15s at 72 °C.

Analysis H_2O_2 in plant tissues

After through washing, the fresh roots and leaves, tissues were crushed in 0.1 % trichloroacetic acid [61] with the aid of mortar and pestle and centrifuged for 15 min at 10,000 rpm. The supernatant was then mixed with potassium iodide (1M) and phosphate buffer (10 mM, ph 7.0) and kept for 1 h in the dark. After that, the optical density (OD) was measured at 390 nm by Genesys 10S UV–VIS spectrophotometer (Thermo Scientific, USA).

Root and shoot NO concentration analysis

The concentration of nitric oxide (NO) in maize roots and leaves was measured on the basis of alterations in hemoglobin absorption and subsequent transformation from oxyhemoglobin (HbO2) to methemoglobin (metHb) in the presence of NO [62]. Plant tissue samples were homogenized in 1 ml of cooled NO buffer containing 0.1 M sodium acetate, 1 M NaCl, and 1 percent (w/v) ascorbic acid (pH 6.0). The admixture was then centrifuged for 5 min (10,000 rpm) at 4 °C, and the supernatants are transmitted to a fresh tube. Subsequently, the HbO_2 solution stock (5 mM) was added to the samples and incubated for 5 min at room temperature. The transmission rate of HbO_2 to metHb was assessed at 401 nm.

Estimation of total soluble protein and sugar in plant tissues

The concentration of total soluble protein in root and leaf was estimated by measuring the optical density at 595nm in a GENESYS 10S UV–Vis spectrophotometer (Thermo Scientific, USA). Using different concentrations of bovine serum albumin (BSA) a calibration curve was prepared from which the concentration of the unknown sample was calculated [49]. Besides, the total soluble sugar was estimated in root and shoot as described earlier [50].

Elemental analysis in tissue and grain

Wheat seeds were ground by blander and 2 g powder were weighed. In a glass beaker, 2 g of samples were then taken and 5 ml of HNO_3 and 2 ml of H_20_2 were added and heated in a microwave oven for 1 min. The concentrations of Mn, Fe and Zn Flame Atomic Absorption Spectroscopy (AAS) connected to an ASC-6100 auto-sampler air-acetylene atomization gas mixture device (Model No. AA-6800, Shimadzu) then estimated the concentrations of Mn, Fe and Zn. A standard solution was also made from their subsequent stock solutions for Fe, Mn and Zn [50]. The remaining flour was stored for further analysis. The concentrations of Fe, Mn and Zn in leaves were calculated as earlier described [60].

Determination of Moisture contents

Moisture content was determined using AOAC (2000) method. Briefly, a piece of aluminum foil was dehydrated in an oven at 105^0c for 1 h and then transferred to the dryer to cool down and the blank foil was weighed. Subsequently, 2 g of flour were weighed through an electronic balance and the sample was spread to uniformity. The samples were dried in an oven at 105^0c for 3 h and shifted to the desiccant to cool down the foil, and its desiccated sample was reweighted. Moisture content was determined by the following formula,

% of moisture= (W1-W2/W1) ×100

Where,

W1 =A sample's weight (g) prior to desiccating

W2 =Weight (g) of the sample after desiccating

Estimation of fat amount

Fat was extracted as earlier described [49]. Fat determination required uninterrupted extraction by n- hexan in a Soxhlet apparatus. Briefly, the container and cover were placed in an oven at 105^0C for 12 h to confirm the weight of the container is unchanging. 10 g of sample were weighed using electric balance and placed into extraction thimble, transfer into soxhlet and poured 250ml n- hexan into the pot and placed it on the boiler veil. Soxhlet apparatus was connected and turned on the water to cool and then turned on the boiler veil. The sample was heated for 6 h (heat rate of 150 drops/min) and evaporated the solvent with the help of a vacuum condenser and incubated the bottle at 80-90^0 C for 1 h. After that, the bottle was transferred to the desiccators and reweighted the bottle and its dried content. Fat content was calculated using the subsequent equation,

Fat content (%) = (Weight of fat/weight of sample) ×100

The fat free sample was stored for the estimation of crude fiber.

Determination of Crude fiber

Two (2) gram fat-free sample was weighted through electric balance and the sample was put into a beaker attached to a condenser. 200 ml hot H_2SO_4 (0.125 M) was added to the beaker and boiled for 30 min. Then the sample was filtered with boiling distilled water and transferred the residue into the beaker. Subsequently, 200 ml hot NaOH (0.313M) was added to the beaker and boiled for 30 min followed by filtration using boiling deionized water, 1% HCL and boiling dH_2O, respectively. Finally, the sample was again filtered twice through 100% ethanol. The sample was dried in an oven at 100^0 C overnight and cooled in a desiccator. The sample was placed into the moisture-free crucible, weighted and ignited for 3 h at 500^0 C in a muffle furnace, cooled and reweighted. Crude fiber content was determined through the following formula,

Crude fiber %= $(W_1 - W_2)/W_0 \times 100$

Where,

W_1= Weight of silica crucible with contents before ashing

W_2= Weight of silica crucible with contents after ashing

W_0= Weight of sample

Estimation of ash content

Ash content was determined using AOAC (2000) method. Briefly, crucibles with the lid were placed into a furnace at 600^0C overnight and cooled down in a desiccator (30 min). The weight of the crucible with lid was measured and 5 g samples were put into it. Afterward, crucibles were burnt in a muffle furnace at 600^0 C overnight, cooled, and reweighed. Ash content was determined using the following equation,

Ash (%) = (Weight of ash/Weight of sample) ×100

Yields

Three panicles were collected from each plot, separating the grain from it, counted the number of grain per panicle and calculated their mean for yield analysis per plots and the total yield of every plot was converted as g/m^2. 1000 grain weight of control and treatments were also taken randomly for comparison.

Estimation of protein in maize grain

Protein concentration was quantified as previously described [50] with slight modification. Standard curve was made using 0 %, .05 %, 0.1%, 0.2%, 0.4% and 0.8% BSA solution. 0.03 g flour was diluted in 10 ml deionized water and 2ml Bradford reagent was added. The sample was centrifuged 2 times at 4000 rpm for 5 min, and the supernatant was taken. The optical density (OD) was taken at 595 nm by a spectrophotometer (Genesys 10S UV–VIS Spectrometer, Thermo Scientific, USA).

Statistical analysis

All the examinations were conducted in three autonomous repetitions. The significance of all groups of data was investigated statistically at $P \leq 0.05$ by one-way ANOVA which was carried out by Duncan's Multiple Range Test (DMRT) by SPSS Statistics 23 software. The graphs existing in this report were prepared via GraphPad Prism 6.

Conclusion

In the current study, the plasma generated from air by low pressure dielectric barrier caused oxidation of the wheat seed surface resulted in rough and cracked seed coat that piloted to the increased water acceptance and the permeability of the seeds, consequently promoting its germination. The active species perceived into the wheat seed caryopses and triggered their biological reactivity, resulting in improved soluble protein activity and supply nutrients to plantlets for growth enhancement. Increase activities of CAT and SOD not only improve plant defense system but also develop adaptive response of wheat plants. In conclusion, plasma treatment shows tremendous promise in practical application in increasing seed germination, different agronomic traits including yield and food value of wheat.

Statement: Formal ethical approval is not required for this experimental work as the plant line used in this work is a cultivated genotype. In addition, the seeds were collected from the local market; hence, permissions and/or licenses for collection of seed specimens are not required complying with relevant institutional, national, and international guidelines and legislation.

References

1. Tittensor, D., Clarke, M. & Gümüş, T. Understanding Islamic aid flows to enhance global humanitarian assistance. *Contemp. Islam* **12**, 193–210 (2018).
2. Zhou, Z., Huang, Y., Yang, S. & Chen, W. Introduction of a new atmospheric pressure plasma device and application on tomato seeds. *Agric. Sci.* **2**, 23–27 (2011).
3. Meng, Y. *et al.* Enhancement of germination and seedling growth of wheat seed using dielectric barrier discharge plasma with various gas sources. *Plasma Chem. Plasma Process.* **37**, 1105–1119 (2017).
4. Alexandratos, N. & Bruinsma, J. World agriculture towards 2030/2050: the 2012 revision. (2012).
5. Nwanze, K. F., da Silva, J. G., Cousin, E. & Frison, E. *Opinion: Food security must be on the table at Rio+ 20*. (2012).
6. Raun, W. R., Johnson, G. V, Westerman, R. L. & Hattey, J. A. Wheat production in the Great Plains of North America. *Wheat. Ecol. Physiol. Yield Determ. Food Prod. Press. New York*

263–275 (1999).
7. Bouman, B. A. M., Humphreys, E., Tuong, T. P. & Barker, R. Rice and water. *Adv. Agron.* **92**, 187–237 (2007).
8. Morison, J. I. L., Baker, N. R., Mullineaux, P. M. & Davies, W. J. Improving water use in crop production. *Philos. Trans. R. Soc. B Biol. Sci.* **363**, 639–658 (2008).
9. Bodelier, P. L. E. & Laanbroek, H. J. Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *FEMS Microbiol. Ecol.* **47**, 265–277 (2004).
10. Zhen, L. & Routray, J. K. Operational indicators for measuring agricultural sustainability in developing countries. *Environ. Manage.* **32**, 34–46 (2003).
11. Blum, A. & Jordan, W. R. Breeding crop varieties for stress environments. *CRC. Crit. Rev. Plant Sci.* **2**, 199–238 (1985).
12. Zhu, X.-G., Long, S. P. & Ort, D. R. Improving photosynthetic efficiency for greater yield. *Annu. Rev. Plant Biol.* **61**, 235–261 (2010).
13. WANG, M., CHEN, Q., CHEN, G. & YANG, S. Effect of Atmospheric Pressure Plasma on Growth and Development of Lettuce [J]. *Acta Agric. Boreali-Sinica* **6**, (2007).
14. Min, W. *et al.* Effects of atmospheric pressure plasma on seed germination and seedling growth of cucumber. *Trans. Chinese Soc. Agric. Eng.* **2007**, (2007).
15. Ling, L. *et al.* Effects of cold plasma treatment on seed germination and seedling growth of soybean. *Sci. Rep.* **4**, 5859 (2014).
16. ZHANG, L.-H. *et al.* Effect of Plasma Treatment of Seed on the Biological Traits and Yield of Rice [J]. *J. Jilin Agric. Sci.* **2**, (2007).
17. Ji, S.-H. *et al.* Effects of high voltage nanosecond pulsed plasma and micro DBD plasma on seed germination, growth development and physiological activities in spinach. *Arch. Biochem. Biophys.* **605**, 117–128 (2016).
18. XU, D. *et al.* Effect of Plasma Treatment of Different Radiation Intensity on Biological Traits, Yield and Output Value of Peanut Seed [J]. *Heilongjiang Agric. Sci.* **12**, (2010).
19. FANG, X. *et al.* Analysis of effect on eggplant yield and value by treating seeds with plasma [J]. *J. Jilin Agric. Sci.* **4**, (2009).
20. FANG, X., BIAN, S. & XU, K. Study on Maize Seeds Treated with Plasma to Influence Biological Properties and Yield of Maize [J]. *J. Maize Sci.* **4**, (2004).
21. Ling, L., Jiangang, L., Minchong, S., Chunlei, Z. & Yuanhua, D. Cold plasma treatment enhances oilseed rape seed germination under drought stress. *Sci. Rep.* **5**, 13033 (2015).
22. Alexieva, V., Sergiev, I., Mapelli, S. & Karanov, E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant. Cell Environ.* **24**, 1337–1344 (2001).
23. Orozco-Cárdenas, M. L. & Ryan, C. A. Nitric oxide negatively modulates wound signaling in tomato plants. *Plant Physiol.* **130**, 487–493 (2002).
24. Sun, M. & Zigman, S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Anal. Biochem.* **90**, 81–89 (1978).
25. Rahman, M. M. *et al.* Mechanisms and signaling associated with LPDBD plasma mediated growth improvement in wheat. *Sci. Rep.* **8**, (2018).
26. Sajib, S. A. *et al.* Plasma activated water: the next generation eco-friendly stimulant for enhancing plant seed germination, vigor and increased enzyme activity, a study on black gram (Vigna mungo L.). *Plasma Chem. Plasma Process.* **40**, 119–143 (2020).

27. HE, R. *et al.* Preliminary study of effect of atmospheric pressure plasma on the germination and seedling of Andrographis paniculata [J]. *Guangdong Agric. Sci.* **16**, (2011).
28. Jiayun, T. *et al.* Effects of atmospheric pressure air plasma pretreatment on the seed germination and early growth of Andrographis paniculata. *Plasma Sci. Technol.* **16**, 260 (2014).
29. Zhao, C. *et al.* Field warming experiments shed light on the wheat yield response to temperature in China. *Nat. Commun.* **7**, 1–8 (2016).
30. Ortiz, R. *et al.* Climate change: can wheat beat the heat? *Agric. Ecosyst. Environ.* **126**, 46–58 (2008).
31. Foyer, C. H., Lelandais, M. & Kunert, K. J. Photooxidative stress in plants. *Physiol. Plant.* **92**, 696–717 (1994).
32. Hameed, A., Shah, T. M., Atta, B. M., Haq, M. A. & Sayed, H. Gamma irradiation effects on seed germination and growth, protein content, peroxidase and protease activity, lipid peroxidation in desi and kabuli chickpea. *Pakistan J. Bot.* **40**, 1033–1041 (2008).
33. Cakmak, I. & Horst, W. J. Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (Glycine max). *Physiol. Plant.* **83**, 463–468 (1991).
34. Agarwal, S. Increased antioxidant activity in Cassia seedlings under UV-B radiation. *Biol. Plant.* **51**, 157–160 (2007).
35. Dobrynin, D., Fridman, G., Friedman, G. & Fridman, A. Physical and biological mechanisms of direct plasma interaction with living tissue. *New J. Phys.* **11**, 115020 (2009).
36. Meiqiang, Y., Mingjing, H., Buzhou, M. & Tengcai, M. Stimulating effects of seed treatment by magnetized plasma on tomato growth and yield. *Plasma Sci. Technol.* **7**, 3143 (2005).
37. Dhayal, M., Lee, S.-Y. & Park, S.-U. Using low-pressure plasma for Carthamus tinctorium L. seed surface modification. *Vacuum* **80**, 499–506 (2006).
38. Sera, B. *et al.* Influence of plasma treatment on wheat and oat germination and early growth. *IEEE Trans. Plasma Sci.* **38**, 2963–2968 (2010).
39. Šerá, B., Gajdová, I., Šerý, M. & Špatenka, P. New physicochemical treatment method of poppy seeds for agriculture and food industries. *Plasma Sci. Technol.* **15**, 935 (2013).
40. Kyzek, S. *et al.* Cold atmospheric pressure plasma can induce adaptive response in pea seeds. *Plasma Chem. Plasma Process.* **39**, 475–486 (2019).
41. Grzegorzewski, F., Rohn, S., Kroh, L. W., Geyer, M. & Schlüter, O. Surface morphology and chemical composition of lamb's lettuce (Valerianella locusta) after exposure to a low-pressure oxygen plasma. *Food Chem.* **122**, 1145–1152 (2010).
42. Filatova, I. *et al.* The effect of plasma treatment of seeds of some grain and legumes on their sowing quality and productivity. *Rom. J. Phys* **56**, 139–143 (2011).
43. Berry, J. A. & Downton, W. J. S. Environmental regulation of photosynthesis. *Photosynthesis* **2**, 263–343 (1982).
44. Jiang, J., Jiangang, L. I. & Yuanhua, D. Effect of cold plasma treatment on seedling growth and nutrient absorption of tomato. *Plasma Sci. Technol.* **20**, 44007 (2018).
45. Ozaki, K. *et al.* Enrichment of sugar content in melon fruits by hydrogen peroxide treatment. *J. Plant Physiol.* **166**, 569–578 (2009).
46. Wu, Z. H., Chi, L. H., Bian, S. F. & Xu, K. Z. Effects of plasma treatment on maize seeding resistance. *J Maize Sci* **15**, 111–113 (2007).

47. Soriano, D., Alvarado-López, S., Zúñiga-Sánchez, E., Orozco-Segovia, A. & Gamboa-deBuen, A. Analysis of nitrogen seed reserves of ten tree species of the tropical dry forest. *South African J. Bot.* **97**, 149–153 (2015).
48. Monaco, T. A. *et al.* Nitrogen effects on seed germination and seedling growth. *Rangel. Ecol. Manag. Range Manag. Arch.* **56**, 646–653 (2003).
49. Zhao, D., Reddy, K. R., Kakani, V. G. & Reddy, V. R. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. *Eur. J. Agron.* **22**, 391–403 (2005).
50. Couée, I., Sulmon, C., Gouesbet, G. & El Amrani, A. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J. Exp. Bot.* **57**, 449–459 (2006).
51. Llorens, N., Arola, L., Bladé, C. & Mas, A. Effects of copper exposure upon nitrogen metabolism in tissue cultured Vitis vinifera. *Plant Sci.* **160**, 159–163 (2000).
52. Ikhtiar, K. & Alam, Z. Nutritional composition of Pakistani wheat varieties. *J. Zhejiang Univ. Sci. B* **8**, 555–559 (2007).
53. Saeid, A. *et al.* Comparative studies on nutritional quality of commercial wheat flour in Bangladesh. *Bangladesh J. Sci. Ind. Res.* **50**, 181–188 (2015).
54. Baljeet, S. Y., Ritika, B. Y. & Roshan, L. Y. Studies on functional properties and incorporation of buckwheat flour for biscuit making. *Int. Food Res. J.* **17**, (2010).
55. Mepba, H. D., Eboh, L. & Nwaojigwa, S. U. Chemical composition, functional and baking properties of wheat-plantain composite flours. *African J. Food, Agric. Nutr. Dev.* **7**, (2007).
56. Jamal, M. *et al.* PHYSIOCHEMICAL AND NUTRITIONAL EVALUATION OF SELECTED PAKISTANI WHEAT VARIETIES. (2006).
57. Teklić, T., Lončarić, Z., Kovačević, V. & Singh, B. R. Metallic trace elements in cereal grain–a review: how much metal do we eat? *Food Energy Secur.* **2**, 81–95 (2013).
58. RAMOS, A. C. *et al.* Ecophysiology of iron homeostasis in plants. (2017).
59. Goud, P. B. & Kachole, M. S. Antioxidant enzyme changes in neem, pigeonpea and mulberry leaves in two stages of maturity. *Plant Signal. Behav.* **7**, 1258–1262 (2012).
60. Almeselmani, M., Deshmukh, P. S., Sairam, R. K., Kushwaha, S. R. & Singh, T. P. Protective role of antioxidant enzymes under high temperature stress. *Plant Sci.* **171**, 382–388 (2006).
61. Zelinová, V., Mistrík, I., Paľove-Balang, P. & Tamás, L. Peroxidase activity against guaiacol, NADH, chlorogenic acid, ferulic acid and coniferyl alcohol in root tips of Lotus japonicus and L. corniculatus grown under low pH and aluminium stress. *Biologia (Bratisl).* **65**, 279–283 (2010).
62. Sidik, M. A. B. *et al.* Effects of Cold Plasma Treatment on the Growth Rate of Corn and Eggplant Plants. *Proc. 2018 Int. Conf. Electr. Eng. Comput. Sci. ICECOS 2018* **17**, 441–446 (2019).

Acknowledgments
We are grateful to Department of Glass and Ceramic Engineering (GCE), Rajshahi University of Engineering & Technology (RUET), Rajshahi-6205, Bangladesh.
Author contributions
Mahedi Hasan, Sohanur Rahman Sohan and Md. Abu Reza conceptualized the whole project. Mahedi Hasan and Sohanur Rahman Sohan performed most of the experiments. Forhad Hossain, Md. Mahmudul Hasan Maruf and Masum Miah was actively involved in different experiments. Mamunur Rashid Talukder, Md. Mamunur Rashid performed seed treatment by plasma technology. Md. Moinuddin was involved in experimentation of nutritional properties. Khandaker Md. Khalid-Bin-Ferdaus, Salek Ahmed Sajib contributed in methodology, software analysis. Ahmad Humayun Kabir contributed in biochemical analysis and qPCR. Mona M. Elseehy and Ahmed M. El-Shehawi provided financial support and reviewing manuscript. Md. Abu Reza supervised the whole work.
Additional Information
Competing interest: The authors declare that they have no known competing interests.
Data Availability Statement The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.
Funding: The current work were funded by University of Rajshahi, Rajshahi-6205, Bangladesh and Taif University Researchers Supporting Project number (TURSP - 2020/75), Taif University, Taif, Saudi Arabia.

Treatment	Grains per panicle		1000 grains weight		Yield	
	Number	% increased	Grams	% increased	Per m^2	% increased
Control	210±17.78^{a}	-	32±4^{a}	-	214.33±12.50^{a}	-
1 min	247.67±22.5ab^{c}	17.94 %	35±3^{ab}	9.38 %	225.33±19.4^{a}	5.13 %
3 min	270.33±27.96^{bc}	28.73 %	36±3^{ab}	12.5 %	228.33±15.50^{a}	6.53 %
6 min	287±29.46^{c}	36.67 %	39±4.51^{b}	22.92 %	272.33±16.50 ^{b}	27.06 %
9 min	234.33±25.58^{ab}	11.59 %	37.67±3.06^{ab}	17.71 %	245.67±21.22^{ab}	14.62 %
12 min	221.67±23.63^{a}	0.79 %	36.67±3.06^{ab}	14.58 %	238.67±26.31^{ab}	11.35 %

Table 1. Effects of plasma treatment on yield of wheat. Different alphabet indicates a significant difference among mean ± SD (n=3) at P < 0.05 level.

Treatment	Moisture%	Fat%	Crude Fiber%	Ash%	Protein (mg/g)
Control	11.41±0.42^{b}	1.02±0.09^{a}	0.85±0.05^{a}	2.36±0.29^{a}	11.75±0.89^{a}
1 min	11.22±0.89^{b}	1.46±0.13^{b}	0.86±0.02^{a}	2.65±0.52^{a}	11.81±2.39^{a}
3 min	11.02±0.44^{b}	1.57±0.32^{b}	0.87±0.07^{a}	2.34±0.23^{a}	11.56±3.17^{a}
6 min	9.0±0.42^{a}	1.73±0.11^{b}	0.93±0.04^{a}	2.55±0.25^{a}	11.69±1.08^{a}
9 min	11.19±0.63^{b}	1.56±0.10^{b}	0.87±0.07^{a}	2.57±0.21^{a}	11.85±3.06^{a}
12 min	12.45±0.83^{b}	1.43±0.24^{b}	0.81±0.07^{a}	2.29±0.2^{a}	11.76±0.97^{a}

Table 2. Evaluation of food value. Different alphabet indicates a significant difference among mean ± SD (n=3) at P < 0.05 level.

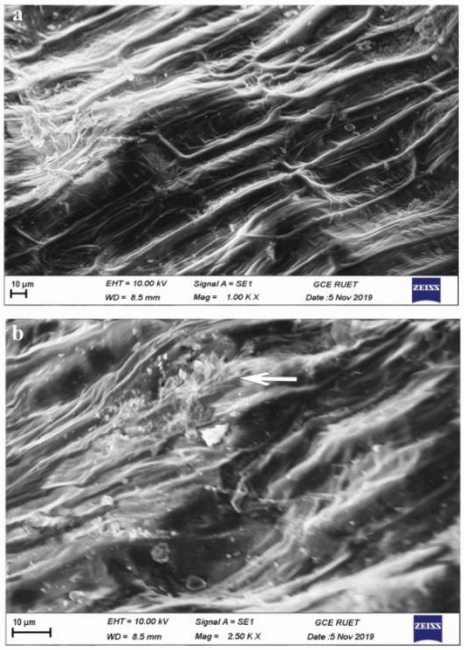

Figure 1. Scanning Electron Microscopy (SEM) images of wheat seed (a) untreated control seed; (b) seeds after 6 min plasma treatment at10.0kV. Crack on the seed coat layer is shown with arrow. Scale bar is 10 µm.

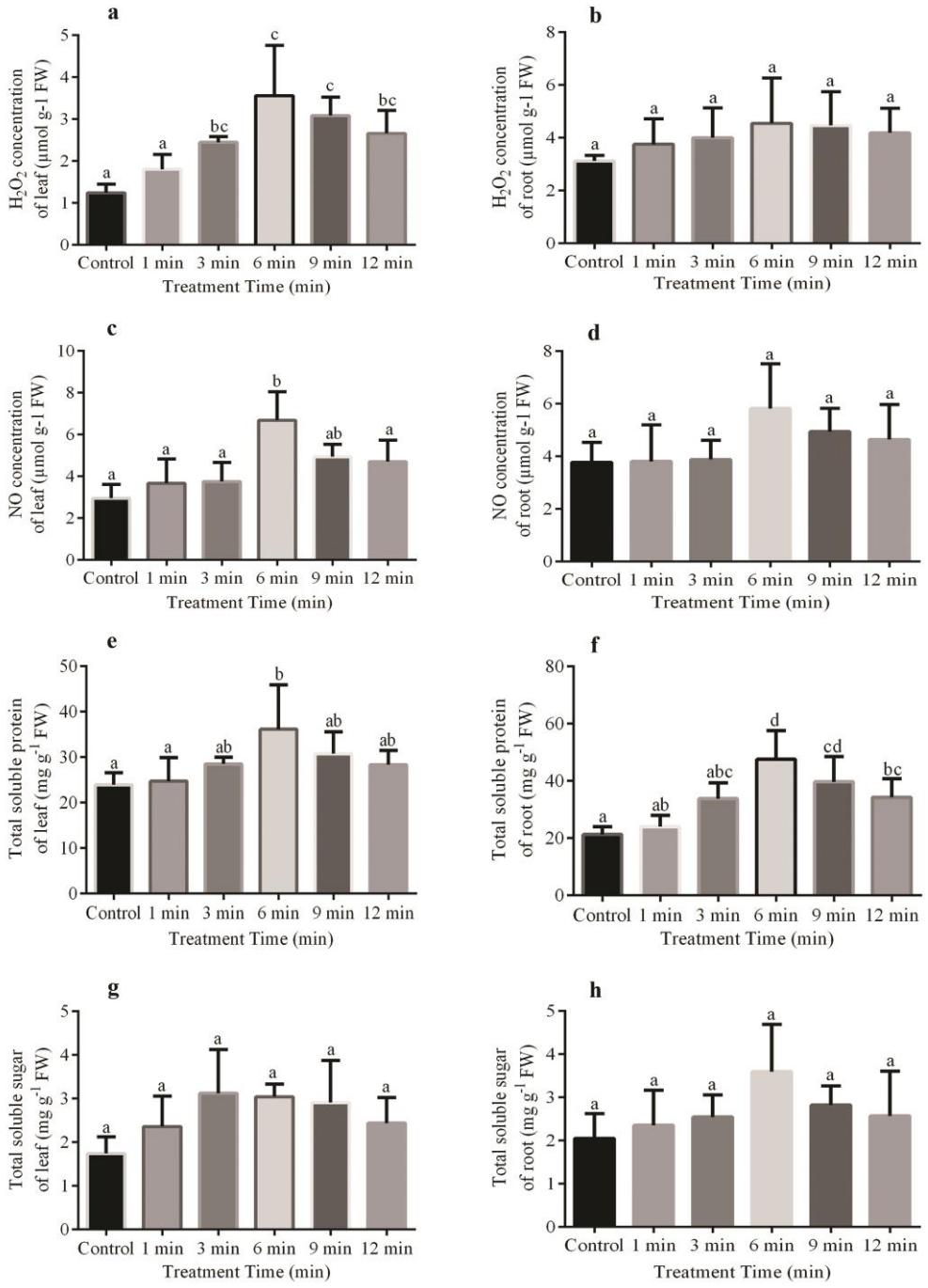

Figure 2. Changes in H_2O_2 activity in (a) leaves,(b) roots; NO activity in (c), leaves (d) roots; contents of soluble protein in (e) leaves,(f) roots; contents of soluble sugar in (g) leaves, (h) roots of wheat plants grown from the seed treated with 0 to 12 min of air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments.

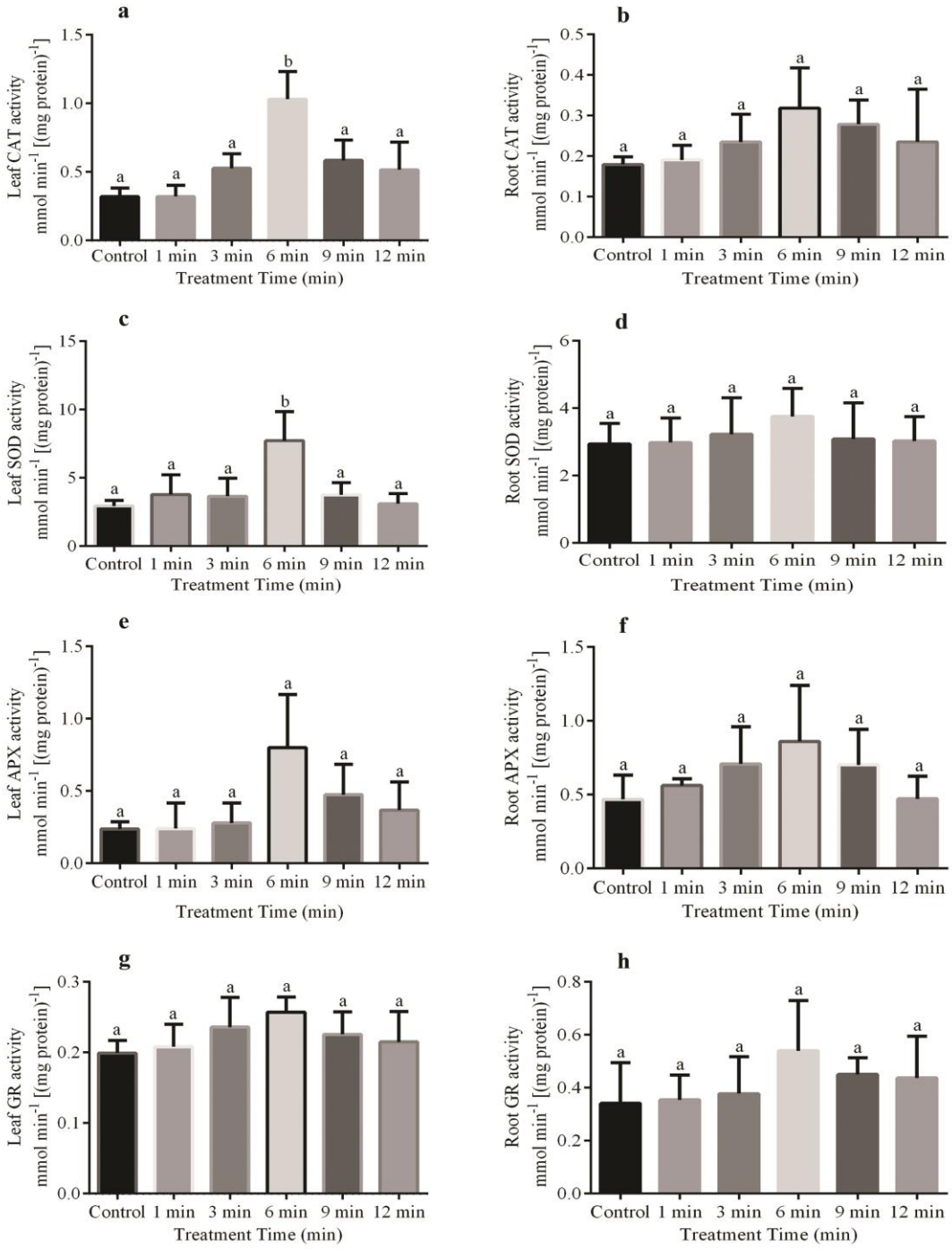

Figure 3. Changes in CAT activity in (a) leaves,(b) roots; SOD activity in (c) leaves (d) roots; APX activity in (e) leaves, (f) roots; GR activity in (g) leaves, (h) roots of wheat plants grown from the seed treated with 0 to 12 min of air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments.

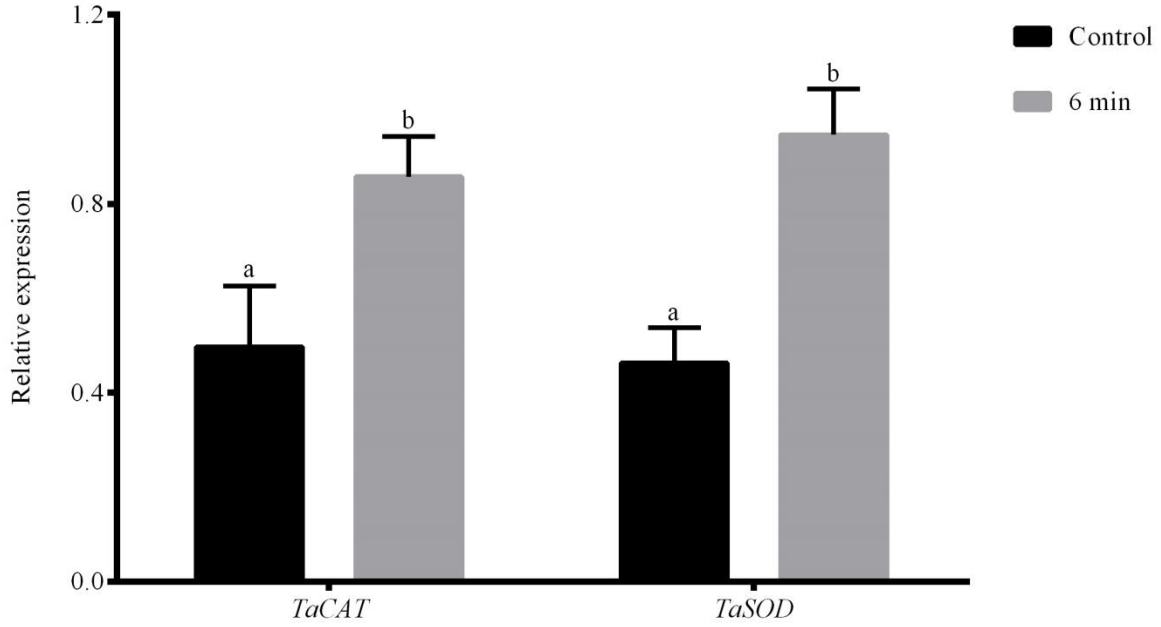

Figure 4. Quantitative study of the expression pattern of *TaCAT* and *TaSOD* in wheat plant roots grown from 6 min air plasma treated seeds. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments.

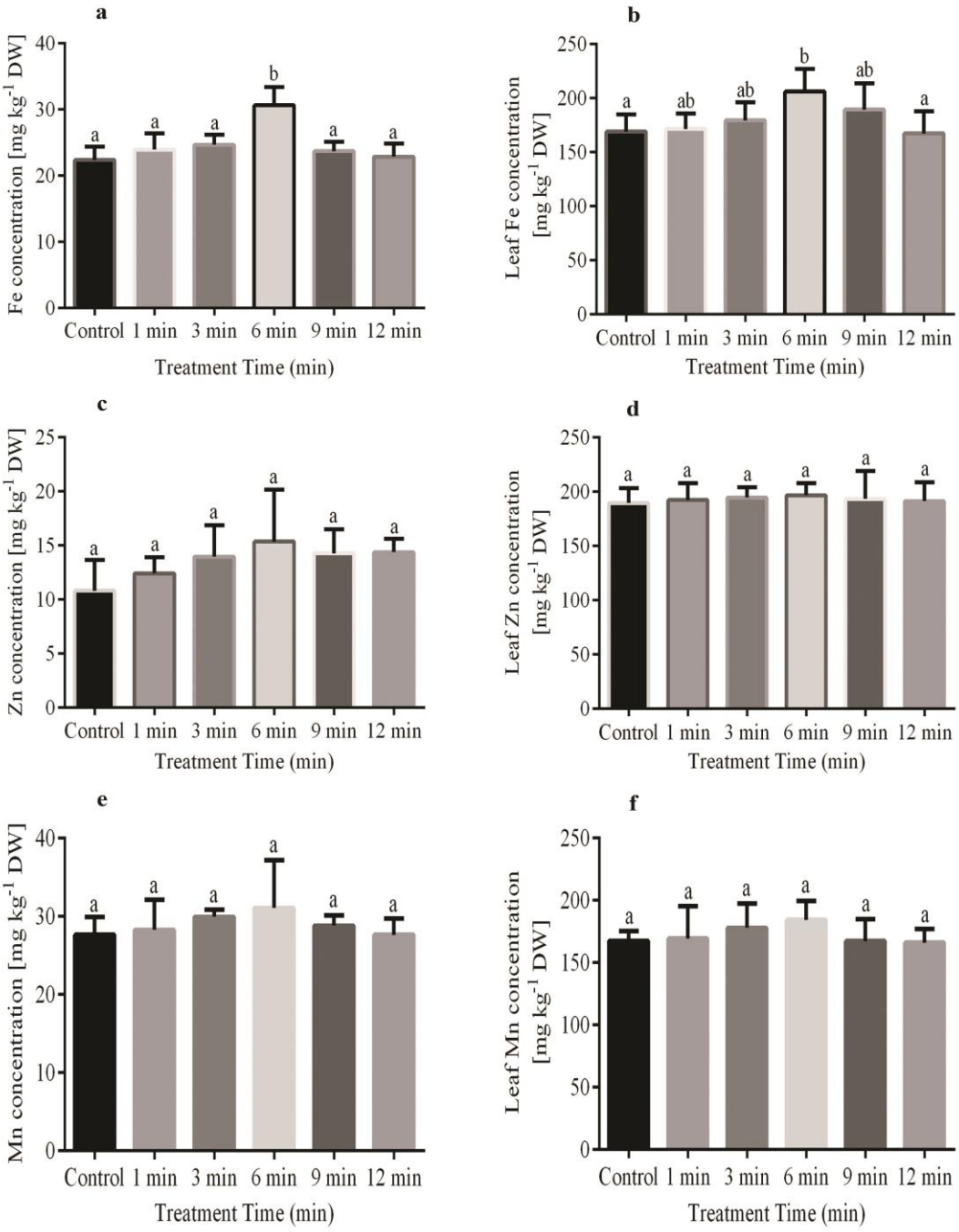

Figure 5. Changes in Fe concentration in (a) grains,(b) leaves; Zn concentration in (c) grains,(d) leaves;Mn concentration in (e) grains, (f) leaves of wheat plants grown from the seed treated with 0 to 12 min air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments.

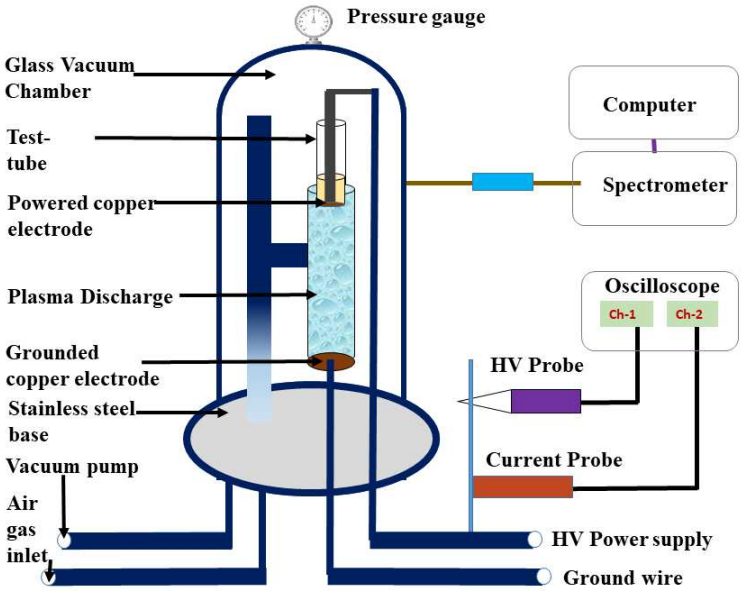

Figure 6. Plasma treatment apparatus.

Figures

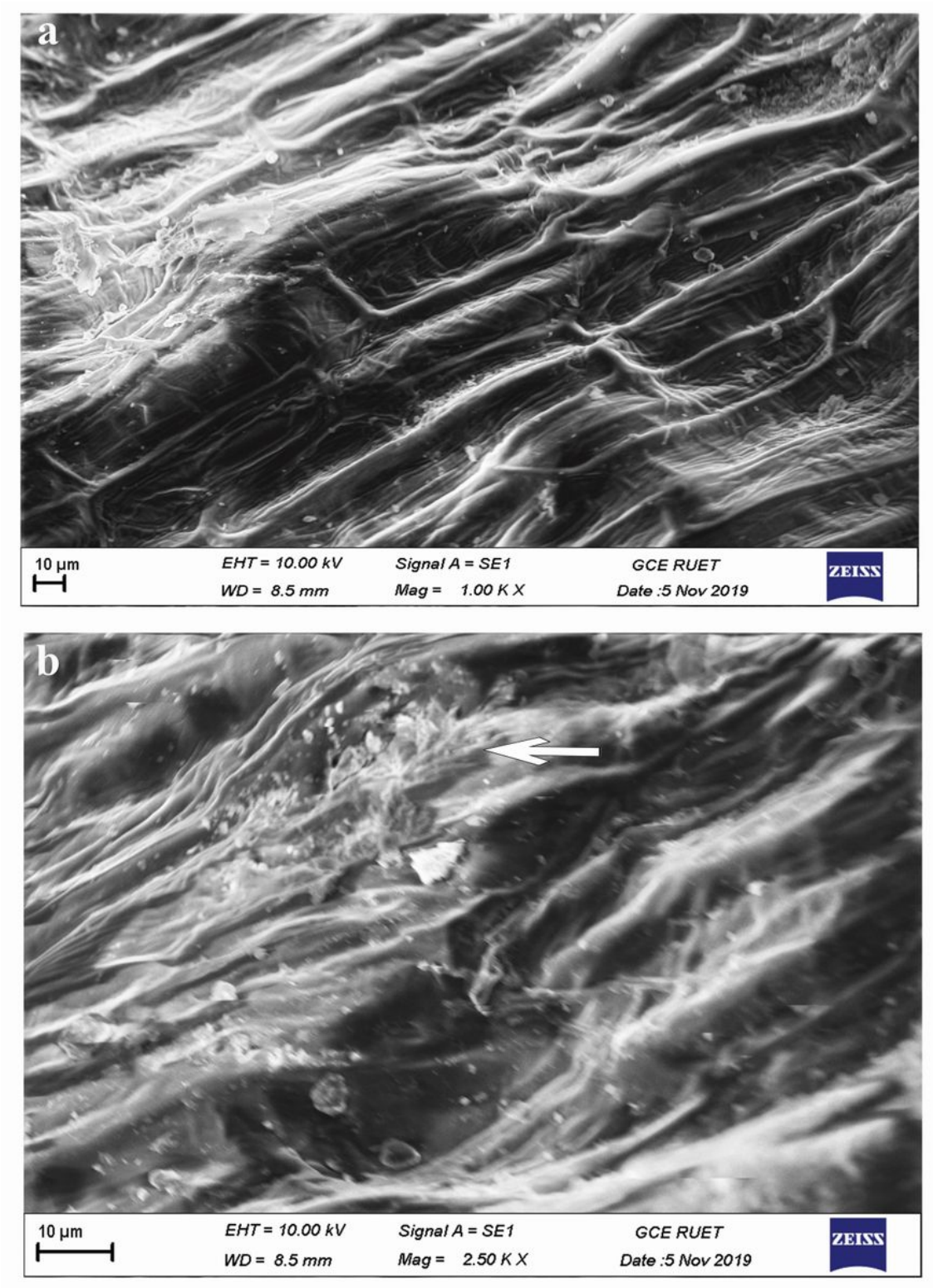

Figure 1

Scanning Electron Microscopy (SEM) images of wheat seed (a) untreated control seed; (b) seeds after 6 min plasma treatment at10.0kV. Crack on the seed coat layer is shown with arrow. Scale bar is 10 µm.

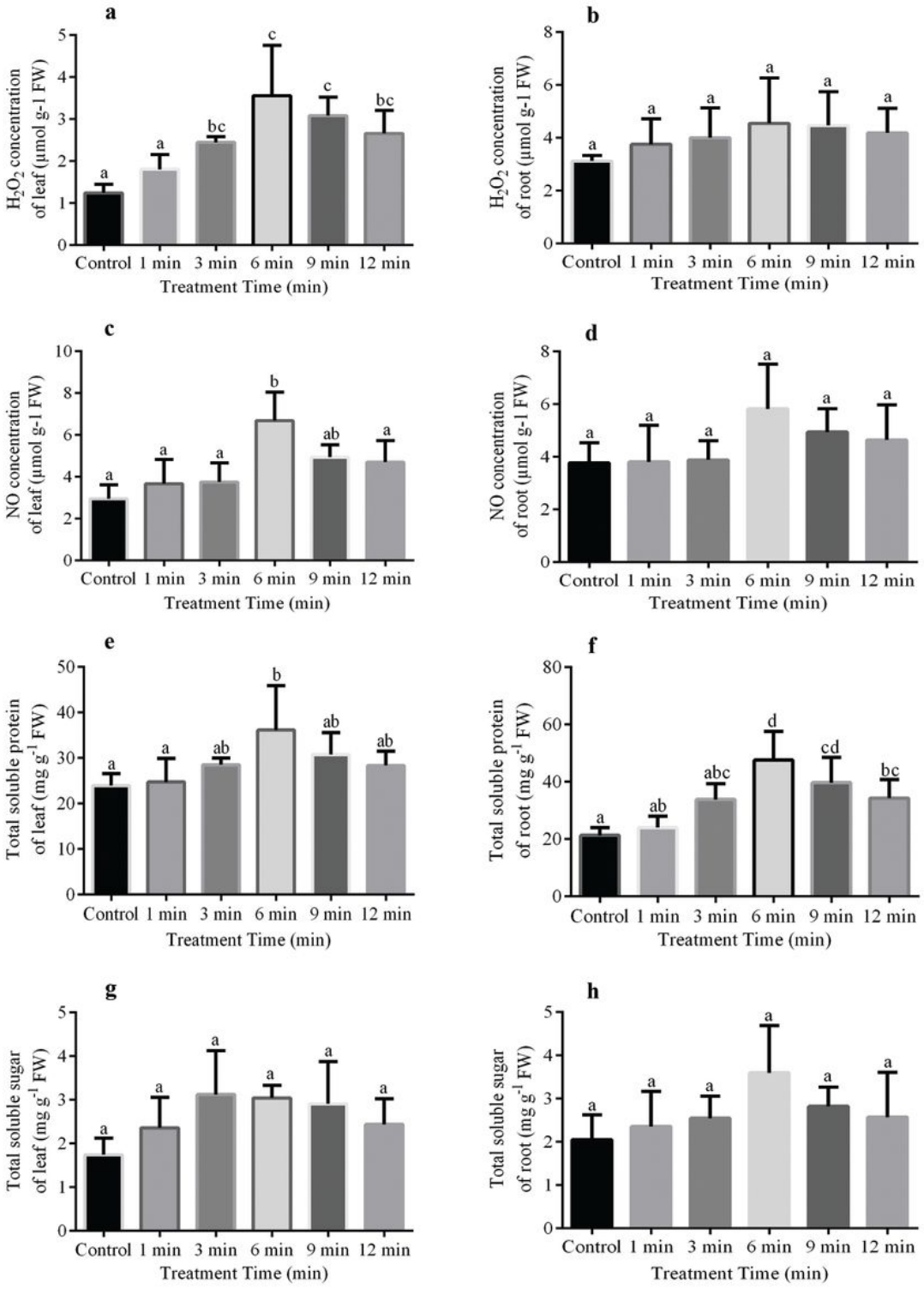

Figure 2

Changes in H2O2 activity in (a) leaves,(b) roots; NO activity in (c), leaves (d) roots; contents of soluble protein in (e) leaves,(f)Changes in CAT activity in (a) leaves,(b) roots; SOD activity in (c) leaves (d) roots; APX activity in (e) leaves, (f) roots; GR activity in (g) leaves, (h) roots of wheat plants grown from the seed treated with 0 to 12 min of air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments. roots; contents of soluble

sugar in (g) leaves, (h) roots of wheat plants grown from the seed treated with 0 to 12 min of air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments.

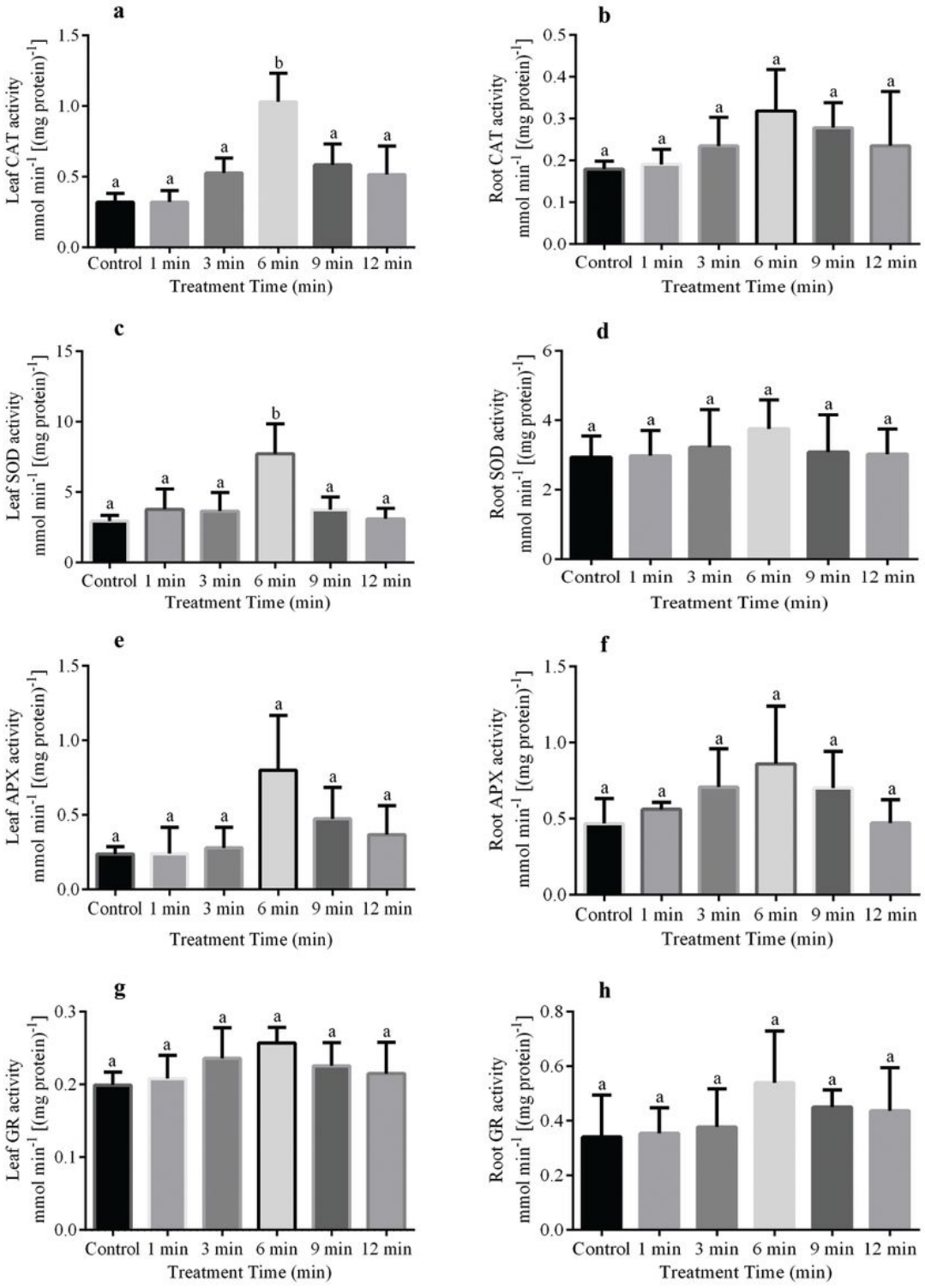

Figure 3

Changes in CAT activity in (a) leaves,(b) roots; SOD activity in (c) leaves (d) roots; APX activity in (e) leaves, (f) roots; GR activity in (g) leaves, (h) roots of wheat plants grown from the seed treated with 0 to

12 min of air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at P < 0.05 level concerning treatments.

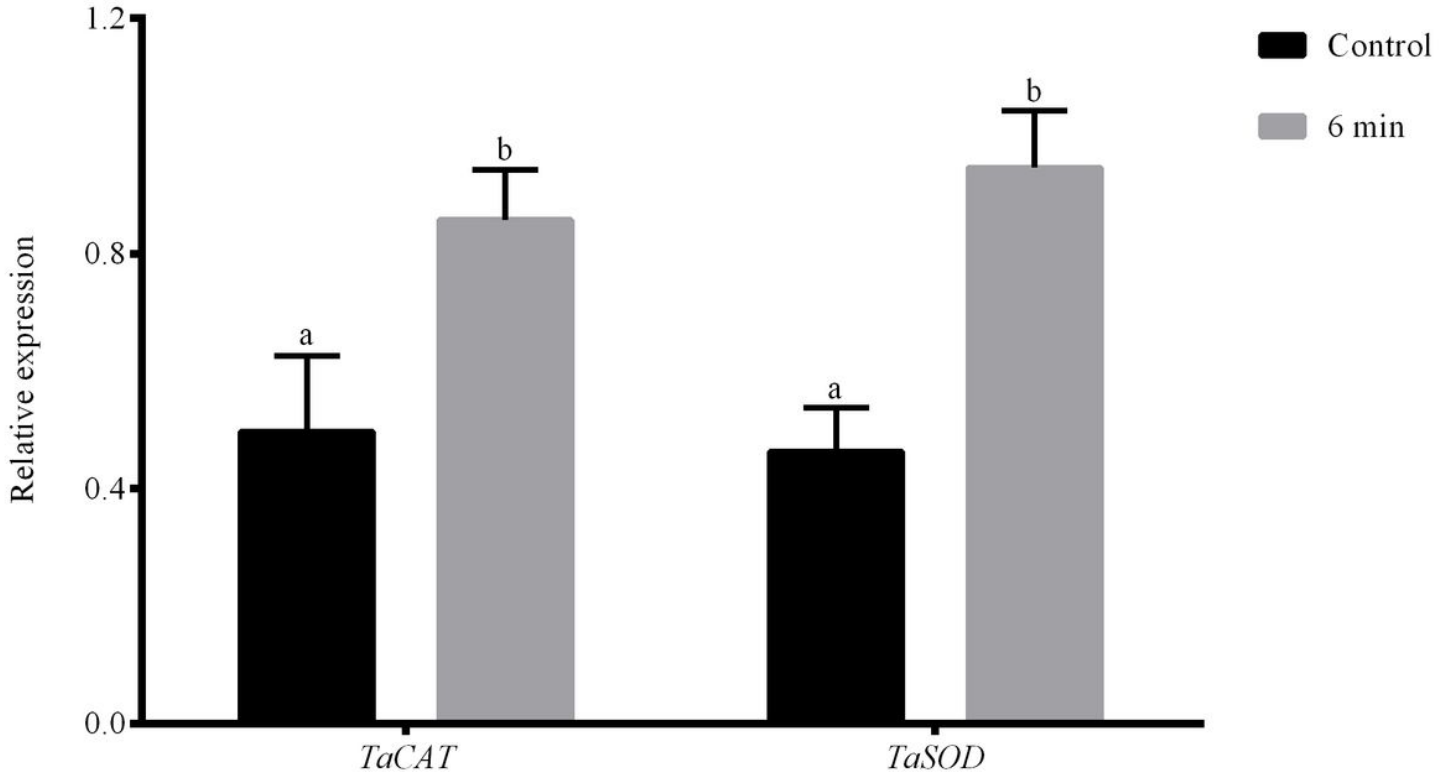

Figure 4

Quantitative study of the expression pattern of TaCAT and TaSOD in wheat plant roots grown from 6 min air plasma treated seeds. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at P < 0.05 level concerning treatments.

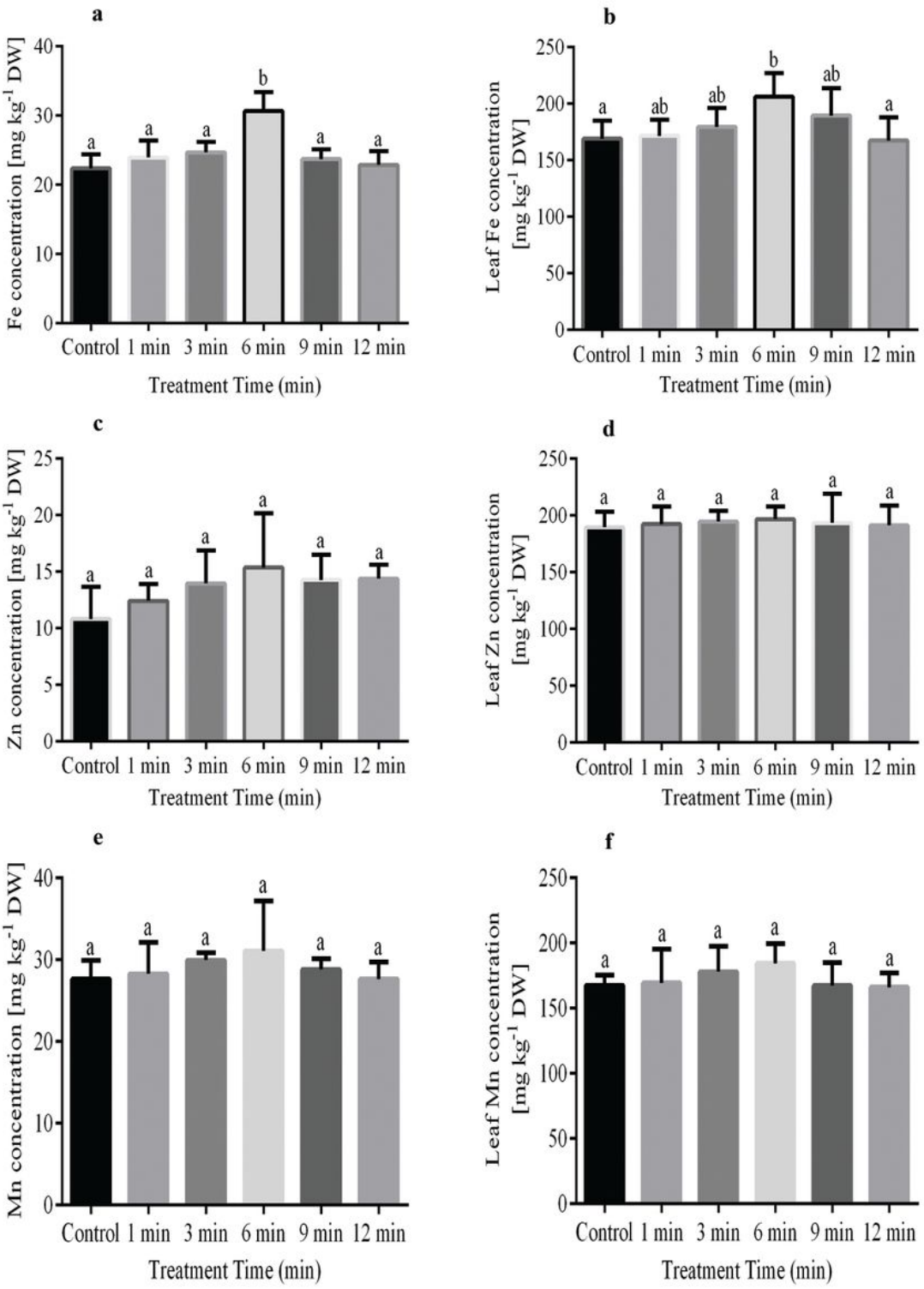

Figure 5

Changes in Fe concentration in (a) grains,(b) leaves; Zn concentration in (c) grains,(d) leaves;Mn concentration in (e) grains, (f) leaves of wheat plants grown from the seed treated with 0 to 12 min air plasma treatment. The alphabets at the top of the bar indicate significant difference among mean ± SD (n=3) at $P < 0.05$ level concerning treatments.

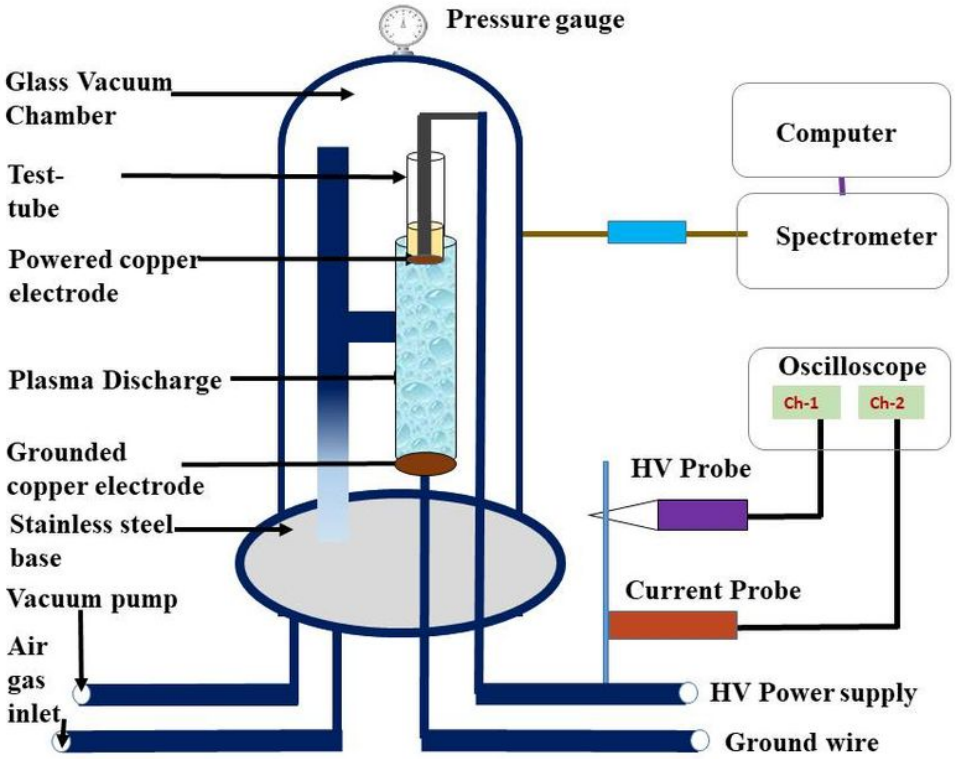

Figure 6

Plasma treatment apparatus.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile.docx