

## **Improvement of Photosynthesis and Photosynthetic Productivity of Winter Wheat by Cold Plasma Treatment under Haze Condition**

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### **ABSTRACT**

Haze stress has various destructive physical, chemical and physiological effects on the plants. Cold plasma treatment is recognized as a suitable technology to improve germination efficiency of numerous seeds. This study was carried out to evaluate the effect of cold plasma in order to reduce harmful effects of haze on winter wheat (Pishgam cultivar) in the greenhouse. Seeds of wheat were treated with cold air plasma at 50W during different time intervals of 60, 120, 180 and 240 seconds. In the greenhouse, simulation of haze stress with concentration of 1500  $\mu\text{g m}^{-3}$  was performed at 13, 49, 61 and 69 Zadoks scales at agricultural research greenhouse of Tarbiat Modares University. Effects of plasma treatments on quality and quantity of wheat were determined by the rate of photosynthesis, leaf stomatal density, size of stomata, intercellular  $\text{CO}_2$  concentration, transpiration rate, chlorophyll content, Relative Water Content (RWC), stomatal conductance, spike yield, and grain yield. Results indicated a significant difference among plasma treatments and the control. Plasma treatment in 180 seconds ameliorated photosynthesis rate, chlorophyll content, and stomatal conductance by 34, 32, and 93%, respectively, compared with the control. In response to haze stress in plasma treatments at 180s plasma treatment, grain and spike yield were increased by 58 and 75%, respectively, compared with the control plants. The results showed that plasma treatments reduced damage of haze stress on winter wheat.

**Keywords:** Climate change, Dust, Environmental problems, Rate of photosynthesis.

### **INTRODUCTION**

Haze is the accumulation of suspended solids in relatively dry air, causing relative darkness in the air and reducing horizontal or vertical visibility. This phenomenon is one of the most serious environmental problems in different parts of the world (Emami, 2015). Haze is a trans boundary phenomenon, while exposure to suspended particles in the air accounts for the early death of 500,000 people annually (Iwai *et al.*, 2005). The effects of haze on human health are manifested as the emergence of various diseases (Al-Hurban

and Al-Ostad, 2010). According to Iran Statistical Yearbook of Agriculture (2011), haze was responsible for damage to crops by about 7-17 million tons in 2009. For example, during 2010-11, agricultural products in Khuzestan province (south of Iran) including irrigated and rain-fed wheat, barley and rapeseed underwent damages over 47 Million US Dollars, accounting for the loss of 468k tons of agricultural products. With the onset of drought, in summer of 2012, haze storms and planting restrictions on 38,000 ha of land in the Karkheh River basin caused 50 Million US Dollars losses to agriculture in this area and affected the livelihoods of 70,000

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farmers, and even led to the migration of many farmers (Iran statistical yearbook, 2011).

Stress and, consequently, plant death occurs when adverse conditions have a negative effect on plant metabolism or inhibit plant growth and development (Kafi *et al.*, 2014; Torabian *et al.*, 2016). The human footprint in every aspect of ecosystem changing and especially in deforestation, destruction of biota and other ecosystem components are being attributed to increase of haze pollution (Grantz *et al.*, 2003). Previous studies have shown that haze, due to its low diameter, penetrates easily into interstitial tissues of plants and reacts with interstitial water, resulting in negative effects on plant metabolism and growth (Sett, 2017). Haze precipitates have numerous physical, chemical and physiological effects on the plant's foliage, including high leaf temperatures (Zia-Khan *et al.*, 2015), increase in pathogens of leaf surface (Wang *et al.*, 2014), decrease in photosynthesis (Xue *et al.*, 2017), stomatal closure, increase in transpiration rate, and loss of dry weight (Rahul and Jian, 2014) and decrease in crop yields (Nanos and Ilias, 2007). Recent studies have shown that the presence of heavy metals in haze enhances damages to cell and intracellular enzymes, influencing the physiological functions of the plant (Jilili *et al.*, 2010).

Seed priming is a technique of seed enhancement, which improves seed performance by rapid and uniform germination, normal and vigorous seedlings, resulting in faster and better germination and emergence of different crops (Khazaei *et al.*, 2013). This also helps seedlings to grow under stress conditions (Ashraf and Foolad, 2005). Priming helps seeds with repairing and building up of nucleic acids, increased synthesis of proteins as well as the repairing of membranes, and enhances the activities of anti-oxidative enzymes in treated seeds (Wang *et al.*, 2003).

The fourth state of matter, which is called Plasma, consists of different oxygen radicals, charged particles, ionized gases, radicals and free electrons (Dobrynin *et al.*, 2009). Plasmas are classified in two categories: thermal or non-thermal. Cold plasma is a non-thermal technology, which can be generated under atmospheric and low-pressure situations (Hertwig *et al.*, 2015). Recent studies showed the cold plasma poses stimulating effects on plants

seeds (Laroussi, 2002). For example, the cold plasma in soybean with a power of 80W increased germination by 14.66% and seedling by 63.33% (Li *et al.*, 2014). Plasma treatments improved the plant's ability to cope with abiotic and biotic stresses, such as drought stress (Zhou *et al.*, 2012) and plant disease (Jiang *et al.*, 2014). This research was conducted to improve the nutritional conditions of plant through cold plasma in order to maintain and increase the yield of wheat in conditions of climate change caused by haze.

## MATERIALS AND METHODS

### Treatment Conditions

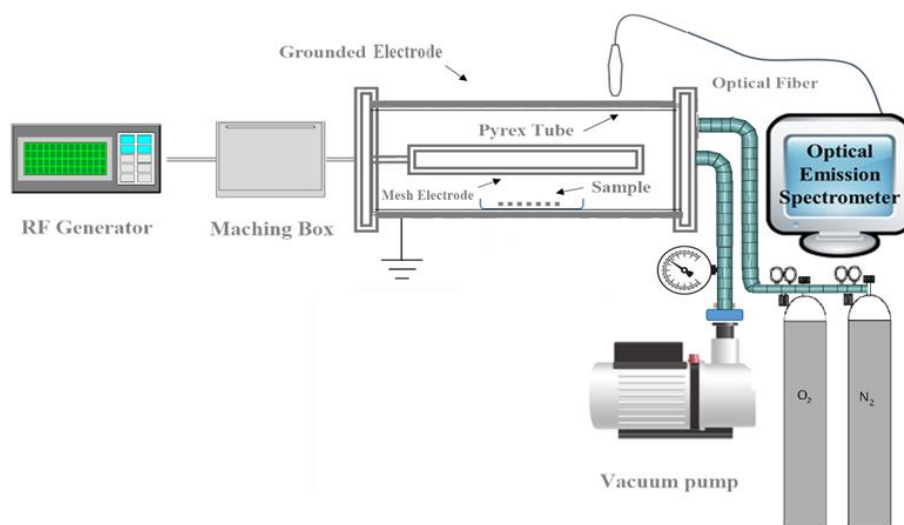
Healthy and uniform seeds were selected and exposed to inductive air plasma discharge under the following parameters: The experimental setup is shown in Figure 1. The plasma was applied by Radio-Frequency (RF) plasma reactor operated with air at 13.56 MHz. The vacuum chamber was made by a cylindrical Pyrex tube with inner diameter of 80 mm and length of 300 mm. The outside of the Pyrex tube was grounded by metallic mesh. The aluminum power electrode was fixed at the center of cylinder (50 mm in width and 100 mm in length). The sample was placed over the Pyrex tube. The gap between the power electrode and the sample was 40 mm. The seeds were exposed to plasma flow at 60, 120, 180, and 240 seconds.

### Plant Material

Seeds of *Triticum aestivum* L. cv. Pishgam, were obtained from the Seed and Plant Improvement Institute, Karaj, Iran.

### Experimental Design

The experiment was carried out in randomized complete block design with three replications, in a research greenhouse situated in Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran (35° 41' N, 51° 10' E, altitude 1,215 m), between September 2016 and May



**Figure 1.** Process of treating wheat seeds by the RF plasma device.

2017. There were five treatments of plasma flow at (p1) 60, (p2) 120, (p3) 180, (p4) 240 seconds, and the control. Three pots for each treatment with a diameter of 18 cm and a height of 14 cm were used. Each pot was filled with sandy loam soil mixed with rotten animal manure at a ratio of 1:4. After irrigation to field capacity, six seeds of the leading cultivar were cultivated in the first stage. After germination and appearance of two leaves, three seedlings were maintained in each pot and the rest were removed. Temperature and humidity of the greenhouse were adjusted according to the plant phenological stage. Wheat bushes were exposed daily to 14 hours of light. The greenhouse experiment continued until physiological maturity of the seeds. The plants were exposed to haze for three consecutive hours in four phenological stages including appearance of three leaves, heading, flowering and milk development (at 13, 49, 61, and 70 Zadoks scales).

### Haze Stress Stimulation

A relatively closed environment with conditions similar to greenhouses was prepared to simulate haze. Soil samples used in this study were prepared from areas prone to haze formation and placed in the oven at 105°C for 24 hours to dry completely. The soil

samples were poured into thick cover and beaten using a hammer to separate the soil particles completely. Due to lack of moisture, the soil samples were converted to fine particles (aggregate) by the entering blows. The haze was simulated artificially by passing the samples from a wind transfer tunnel device (Figure 2). The concentration of haze suspended in the air was experimentally adjusted by changing the wind speed of the wind tunnel. The particle counter (176000 A Microhaze Pro Haze Monitor) was used to find out the concentration and distribution of particle size in the air. This device also shows the distribution of particles size. The concentration of particles suspended in the air was considered to be 1,500  $\mu\text{g m}^{-3}$  in line with its mean rate in the last five years on the hazy days of the high restrictive areas (Shahsavani *et al.*, 2012).

### Measurement of Photosynthesis Rate and Intercellular CO<sub>2</sub> Concentration

Indices to evaluate photosynthesis, including photosynthetic rate, transpiration, stomatal conductance, WUE (water use efficiency) and intercellular CO<sub>2</sub> were measured using LICOR 6400XT version 6 (Lincoln, Nebraska). Measurements were taken from samples of

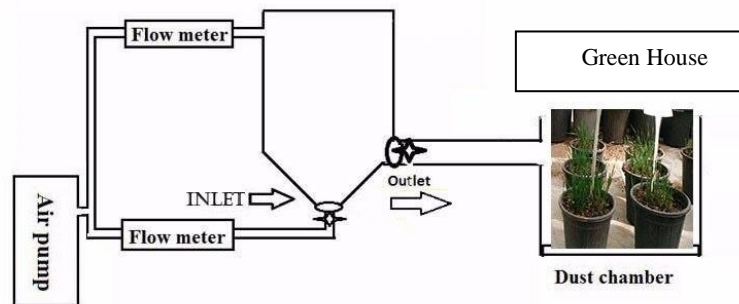


Figure 2. Haze generator and haze chamber used in this study.

young, but developed, leaves of three randomly selected plants from each pot.

#### Measurement of Water Use Efficiency (WUE)

WUE was calculated according to Farquhar and Sharkey (1982). Photosynthetic (A); transpiration (E) rates were measured using LI-COR 6400XT.

$$WUE = A/E \quad (1)$$

#### Measurement of Relative Water Content (RWC)

To measure RWC, flag leaves were used. The fresh weight was immediately recorded after Leaf Excision (FW). The leaves were left in distilled water for 24 hours at 4°C in darkness and the Turgid Weight (TW) was recorded. The Dry Weight (DW) was then measured after 48 hours at 80°C. The RWC were determined following Equation (1) by the method of Teulat *et al.* (2003).

$$RWC = (FW - DW) / (TW - DW) \quad (2)$$

#### Measurement of Chlorophyll Content (Spad Value)

Leaf relative chlorophyll content was measured by chlorophyll meter (SPAD-502, Minolta, Osaka).

#### Measurement of Stomatal Size and Density

The stomatal size and density were determined following Paul *et al.* (2017). The suitable leaf material/plant part were collected, washed gently with running water to remove the haze and debris, and allowed to dry. If peeling of epidermal layer was possible, then it was used directly, otherwise, the suitable replica fluid such as quick fix in a thin and uniform film (by spreading a drop or two of quick fix on the leaf surface) was applied and allowed to dry completely. Then, the replica was gently peeled off with the help of forceps or fingers and placed on the slide in a manner that imprinted surface was on upper side. In order to proper spreading put one or two drops of water/glycerol of replica and cover it with a coverslip. Similar preparations can be made for different leaves for their upper and lower surfaces. Number of stomata present in microscopic view field was recorded for calculating the stomatal density, which was expressed in terms of stomata  $\text{mm}^{-2}$ .

#### Statistical Analysis

All data are presented as the mean value  $\pm$  Standard Error (SE) of three replicates. Statistical analysis was performed using SAS® Version 9.2. Least Significant Difference (LSD) test at the 0.05 probability

level was used to check significant differences between means.

stomatal conductance compared with the control (Figure 4-a).

## RESULTS

### Photosynthesis Rate

There was no significant difference between replications and treatments for wheat photosynthesis rate (Table 1). The effect of cold plasma on photosynthesis of wheat varied with different treatment time levels (Figure 1-a). The p3 and p1 treatments significantly increased the photosynthesis rate by 34 and 31%, respectively, compared with the control, but there was no significant difference between p2 and p4 with the control (Figure 3-a).

### Chlorophyll Content

There was a significant difference between treatments for wheat chlorophyll content (Table 1). The p3 and p2 plasma treatments significantly increased the chlorophyll content by 32 and 27% compared with the control, but there was no significant difference between p1, p4, and the control (Figure 3-b).

### Stomatal Conductance

There was a significant difference between treatments for wheat stomatal conductance (Table 1). Effects of cold plasma on the stomatal conductance of wheat is shown in Figure 4-a. The p3, p4, p2 treatments significantly increased stomatal conductance by 92, 70, and 52%, respectively, compared with the control. The p1 treatment had no significant effect on the

### Mesophyll Conductance

There was a significant difference between treatments for wheat mesophyll conductance (Table 1). The cold plasma improved the mesophyll conductance in p1 and p4 treatments compared with the control, but there was no significant difference between p2 and p3 plasma treatments (Figure 4-b).

### Water Use Efficiency (WUE)

There was a significant difference between treatments for wheat WUE (Table 2). WUE was significantly increased in all plasma treatments under haze stress. WUE was increased in 60s (p1) plasma treatment by 63% compared to control (Figure 5-a).

### Relative Water Content (RWC)

There was a significant difference between treatments for wheat RWC (Table 2). Effect of cold plasma treatment on RWC was similar to WUE and significantly increased in all plasma treatments under haze stress. The highest RWC was in 240s (p4) plasma treatment compared to the control by 28% (Figure 5-b).

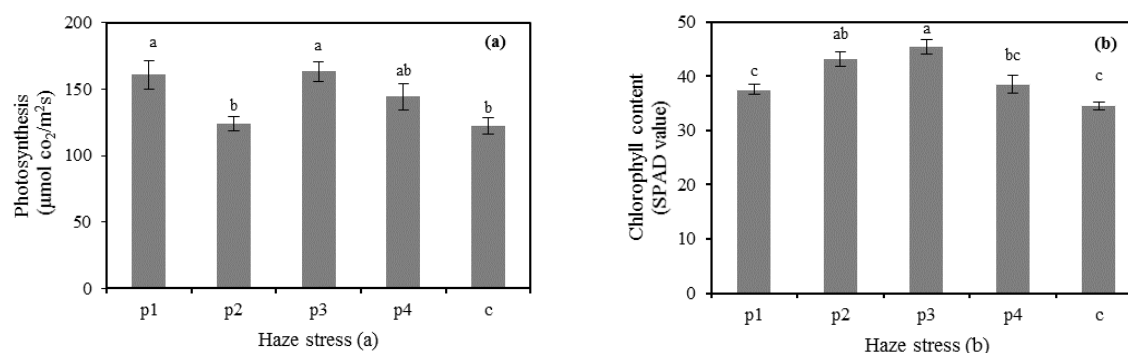
### Leaf Stomatal Density

There was a significant difference between treatments for wheat stomatal density (Table 2). Effect of cold plasma treatment on leaf

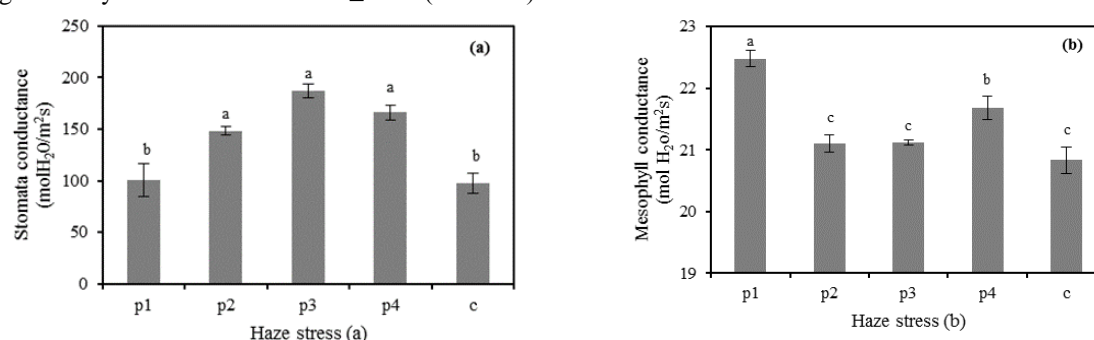
**Table 1.** Analysis of variance of characteristics in wheat under different times of plasma treatment.

Source of Variation	df	Mean square			
		Photosynthesis rate	Chlorophyll content	Stomatal conductance	Mesophyll conductance
Replication	2	17.57	4.66	0.000002	1495.29
Treatments	5	30.35	33.54**	0.000032**	8053.92**
Error	10	16.58	4.44	0.000010	1549.43
CV (%) <sup>a</sup>		10.90	5.21	16.56	18.63

<sup>a</sup> CV: Coefficient of Variation. \*\*: Significant at 1% probability levels, respectively.



**Figure 3.** Effect of cold plasma treatments on photosynthesis rate (a) and chlorophyll content (b) in haze stress. The results are the average of three replications. Different lower case letters on top of bars indicate significantly different means for  $P \leq 0.05$  (LSD test).



**Figure 4.** Effect of cold plasma treatments on stomatal conductance (a) and mesophyll conductance (b) in haze stress. The results are the average of three replications. Different lower case letters on top of bars indicate significantly different means for  $P \leq 0.05$  (LSD test).

stomatal density significantly increased in p4 plasma treatment by 50% compared with the control under haze stress (Figure 6-a).

### Leaf Stomatal Size

There was a significant difference between treatments for wheat stomatal size (Table 2). Effect of cold plasma treatment on stomatal size significantly decreased in all plasma treatments under haze stress. In p1 and p4 plasma treatments, stomatal size was significantly decreased by 43 and 39% compared with the control (Figure 6-b).

### Intercellular CO<sub>2</sub> Concentration

There was a significant difference between treatments for wheat intercellular CO<sub>2</sub>

concentration (Table 3). Effect of cold plasma treatment on intercellular CO<sub>2</sub> concentration significantly increased in all treatments under haze stress condition. The highest intercellular CO<sub>2</sub> concentration plasma caused was in the p2 and p4 plasma treatments, which were increased by 73 and 41%, respectively, compared to the control (Figure 7-a).

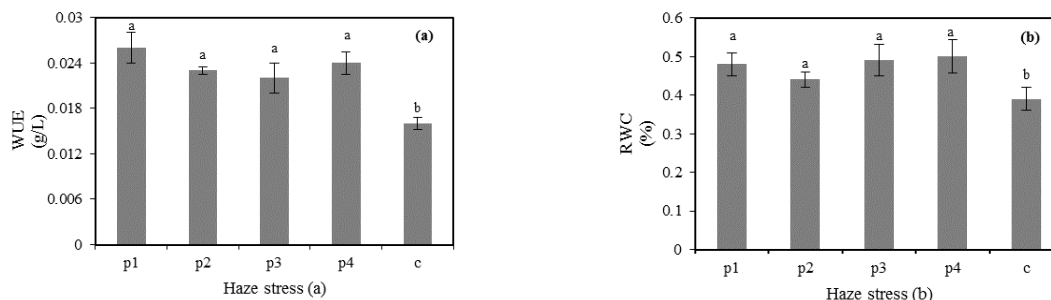
### Transpiration Rate

There was a significant difference between treatments for wheat transpiration rate (Table 3). Effect of cold plasma treatment on transpiration rate significantly increased in all plasma treatments under haze stress condition. The highest transpiration rate plasma caused belonged to the p1 plasma treatment and was increased by 64% compared to the control. The p2, p3 and p4 plasma treatments were in the same statistical group (Figure 7-b).

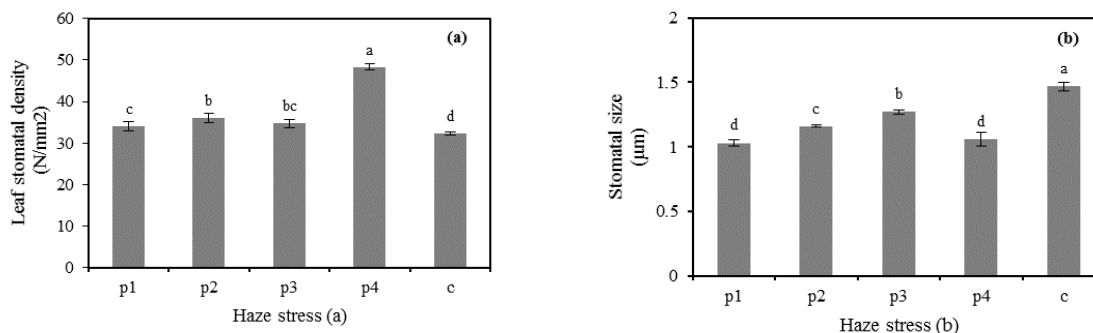
**Table 2.** Analysis of variance of characteristics in wheat under different times of plasma treatment.

Source of Variation	df	Mean square			
		WUE	RWC	Leaf stomatal density	Stomatal size
Replication	2	0.17	0.005	18.60**	0.02**
Treatments	5	0.97**	0.017**	59.29**	1.57**
Error	10	0.17	0.006	1.06	0.003
CV (%) <sup>a</sup>		16.07	16.52	2.85	3.45

<sup>a</sup> CV: Coefficient of Variation. \*\* Significant at 1% probability levels, respectively.



**Figure 5.** Effect of cold plasma treatments on *WUE* (a) and *RWC* (b) in haze stress. The results are the average of three replications. Different lower case letters on top of bars indicate significantly different means for  $P \leq 0.05$  (LSD test).



**Figure 6.** Effect of cold plasma treatments on leaf stomatal density (a) and stomatal size (b) in haze stress. The results are the average of three replications. Different lower case letters on top of bars indicate significantly different means for  $P \leq 0.05$  (LSD test).

### Grain and Spike Yield in Haze Stress and Normal Conditions

**Grain Yield:** The influence of cold plasma on the grain yield of wheat in haze stress is shown in (Figure 8-a). All plasma treatments significantly increased grain yield compared with the control in haze stress condition. The highest grain yield (grams per plant) belonged to the p3 plasma treatment and was 58%

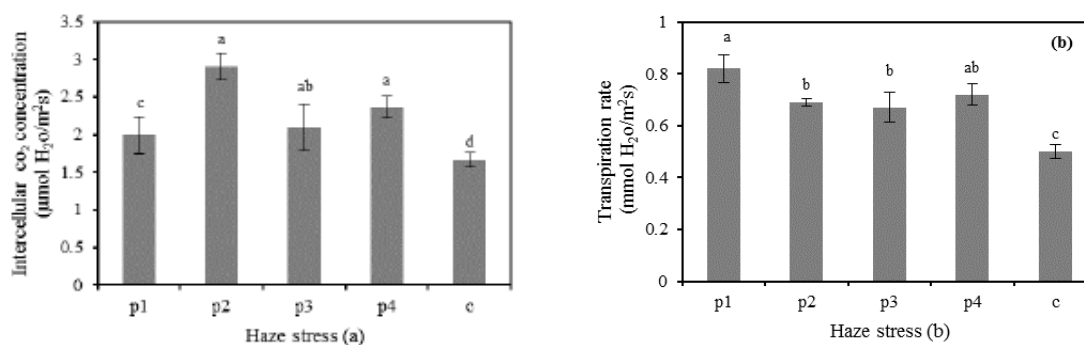
higher compared to the control (Figure 8-a). In no stress condition, treatment of 180s (p3) plasma treatment was significantly higher by 21% compared with the control (Figure 8-b). The lowest grain yield belonged to p4 plasma treatment in no stress condition (Figure 8-b).

**Spike Yield:** Spike yield (grams per plant) in the p3, p4 and p2 treatments significantly increased compared with the control in haze condition. The highest spike yield belonged to the p3 plasma treatment and was 74% higher than the control (Figure 9-a). In normal condition, spike yield of p3, p4, and p2 plasma treatments significantly increased compared

**Table 3.** Analysis of variance of characteristics in wheat under different times of plasma treatment.

Source of variation	df	Mean square	
		Intercellular CO <sub>2</sub> concentration	Transpiration rate
Replication	2	0.17	0.005
Treatments	5	0.97**	0.017**
Error	10	0.17	0.006
CV (%) <sup>a</sup>		16.07	16.52

<sup>a</sup> CV: Coefficient of Variation. \* and \*\*: Significant at 5 and 1% probability levels, respectively.

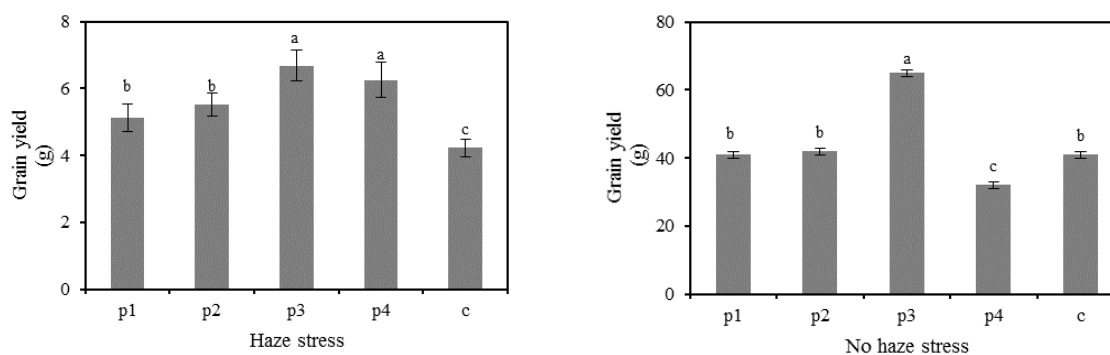


**Figure 7.** Effect of cold plasma treatments on intercellular CO<sub>2</sub> concentration (a) and transpiration rate (b) in wheat under haze stress. The results are the average of three replications. Different lower case letters on top of bars indicate significantly different means for  $P \leq 0.05$  (LSD test).

**Table 4.** Analysis of variance for the main and interaction effects of plasma treatment and haze condition on wheat characteristics.<sup>a</sup>

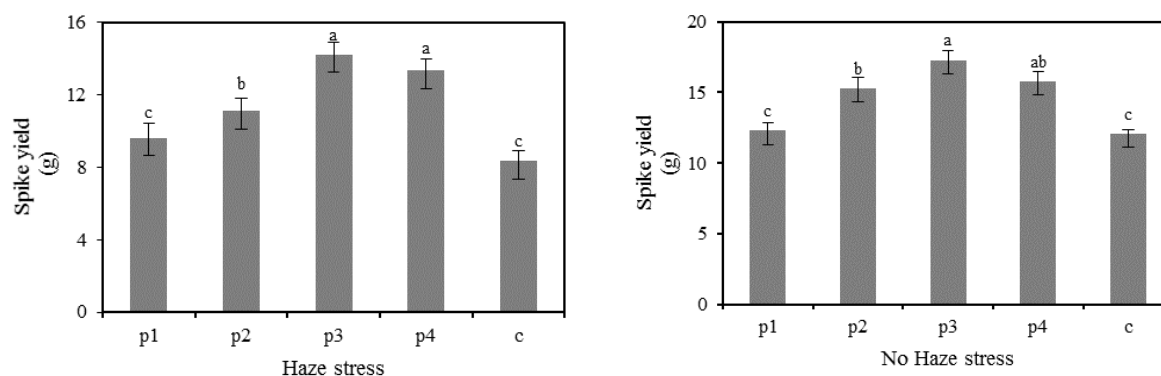
Source of Variation	df	Mean square	
		Grain yield	Spike yield
Replication	2	0.002	0.15
Plasma (P)	4	0.040**	6.29**
Haze (H)	1	0.220**	41.30**
P×H	4	0.009**	2.41**
Error	18	0.001	0.09
CV (%)		9.33	5.12

<sup>a</sup> df.: Degree of freedom; CV: Coefficient of Variation; ns: Not significant at the 0.05 probability level. \* and \*\*: Significant at 5 and 1% probability levels, respectively.



**Figure 8.** Effect of cold plasma treatment on grain yield in haze stress (a) and no stress condition (b). Different lower case letters denote statistical differences between treatment groups at the 5% level according to LSD test.





**Figure 9.** Effect of cold plasma treatment on spike yield in haze stress (a) and no stress condition (b). Different lower case letters denote statistical differences between treatment groups at the 5% level according to *LSD* test.

with the control. The highest spike yield causes by the p3 plasma treatment was increased by 43 % compared to the control (Figure 9-b).

## DISCUSSION

Results showed that cold plasma had a positive effect on the characteristics of the chlorophyll content and net photosynthesis rate of wheat in haze condition. The environmental stress causes reduction in the chlorophyll content. This reduction could be due to destruction of the chloroplast and instability of chlorophyll protein complex (Zia-Khan *et al.*, 2015). Farooq *et al.* (2009) have shown that both chlorophyll a and b decrease during abiotic stress. In contrast, Silvente *et al.* (2012) have not observed any change in the chlorophyll a and b content in stress condition. They also report no significant decrease on chlorophyll a/b ratio in tolerant and sensitive varieties of soybean under stress condition.

It is shown that changes in leaf physiological parameters had direct effect on growth, yield, and development of plants (Proietti and Famiani, 2002). The haze stress causes chloroplast to break down and reduce the amount of chlorophyll. Plants with a higher sensitivity to stress have a more unstable chlorophyll-protein complex (Lawlor and Comic, 2002). Deposition of particles of haze on leaf surface of plants cause reduction in quantity and intensity of light to reach the chloroplast and, consequently, decreases the

photosynthetic pigments (Thompson *et al.*, 1984) and slows down photophosphorylation.

High levels of chlorophyll in plasma treatments can be attributed to increased physiological activity and photosynthesis in plants. The positive correlation between stomatal conductance and chlorophyll content indicates more CO<sub>2</sub> processing (Metwally *et al.*, 2003). Enhanced leaf photosynthesis results in increased phloem sap movement and plant growth, ensuring maximum seed fill, which will deliver the final yield (Hayat and Ahamd, 2007). Other researchers have emphasized the effects of plasma on increasing chlorophyll content in tomato and maize at no stress condition (Henselová *et al.*, 2012; Jiang *et al.*, 2014). However, Šerá *et al.* (2009) reported no significant changes in chlorophyll content in plasma treatment on rape seedlings. We observed that cold plasma treatment at 180s increases chlorophyll content and photosynthesis rate by 32 and 34%, respectively, compared with the control in haze condition (Figures 3-a and -b).

Plasma treatment of 180s increased intercellular CO<sub>2</sub> (Figure 7-a), stomatal conductance and mesophyll conductance in haze condition (Figures 4-a and -b). Restriction of reduction in stomatal conductance and mesophyll conductance plays an important role on maintaining photosynthetic activity (Idrees *et al.*, 2010). Under stress environmental conditions, both stomatal and non-stomatal factors (stomatal conductance and mesophyll conductance) can decrease the photosynthetic abilities of plants



(Liu *et al.*, 2007). The reduction of intracellular CO<sub>2</sub> is one of the most important factors limiting photosynthesis. The balance of carboxylation and oxygenation depends on CO<sub>2</sub> to O<sub>2</sub> ratio on the rubisco (ribulose-1, 5-bisphosphate carboxylase/oxygenase) site (Osborne *et al.*, 1998). As the result, reduction in CO<sub>2</sub> and rubisco enzyme causes the replacement of oxygenation reaction (photorespiration) with the process of carboxylation (photosynthesis). Sharifi *et al.* (1997) reported that haze of 10 g m<sup>-2</sup> reduced Photosynthetically Active Radiation (PAR) absorption by 20% in desert shrubs. They found an inverse relationship between the weights of haze per unit leaf surface to absorbance of PAR. Therefore, apart from the closure of the stomatal conductance, another reason for the increase of canopy temperature of the haze treatment is the increased absorbance of the near-infrared solar irradiance. For example, Eller (1977) reported an experimental study in Switzerland and showed a doubling of absorbance in the near-infrared (700–1,350 nm) for hazy leaves compared with the control leaves, which resulted in a decrease in the intracellular CO<sub>2</sub>. It is shown that plasma treatment increases the plant height and, as the result, the plant could compete for more sunlight (Jiang *et al.*, 2014). Higher level of absorption of PAR causes increase in stomatal conductance, chlorophyll content and net photosynthesis rate (Taiz and Zeiger, 2006).

The stomata in plants are the vital gate between plant and environment, which can play a strategic role in responses to environmental stress (Nilson and Assmann, 2007). Several researchers have reported that stomatal density can have key responses to various environmental stresses, such as elevated CO<sub>2</sub> concentration (Woodward, 1987), heat stress (Beerling and Chaloner, 1993), drought (Zhao *et al.*, 2001; Galmes *et al.*, 2007), precipitation change (Yang *et al.*, 2007), and plant density (Zhang *et al.*, 2003). Haze stress altered the structure of the leaves and various authors underlined the reduction of plant growth, as a consequence of haze stress (Gupta and Iqba, 2005). Nevertheless, that treatment which act to resistant under haze stress conditions and despite the observed

modifications they continue to grow and reach maturity or flowering stage (Gostin, 2009). Plasma treatments increased the density of stomatal and decreased the size of stomatal (Figures 6-a and -b). The stomatal density was positively correlated with stomatal conductance, net CO<sub>2</sub> assimilation rate, and Water Use Efficiency (WUE). The present results indicate that high flexibilities in stomatal density and guard cell size will change in response to plasma treatment (Figure 6-a), and this process may be closely associated with photosynthesis and water use efficiency.

Plasma treatments also improved *RWC* and *WUE* (Figures 5-a and -b) of wheat under haze stress condition. Leaf *RWC* is among the best growth/biochemical indices revealing the stress intensity (Alizadeh, 2002). The *RWC* in plants with high resistance against stress is higher than others. Under stress condition, cell membrane is subject to changes such as increase in penetrability and decrease in sustainability (Blokchina *et al.*, 2003). Probably, in these conditions, ability for osmotic adjustment is reduced (Meyer and Boyer, 1981). Increasing *RWC* in haze-stressed plants by application of plasma might be due to the balance between leaf water content and transpiration rates better than other components. This may also be an indicator of the degree to which the tissues and cells are hydrated, which is a crucial factor for optimal physiological functioning.

Water use efficiency is often equated in a simplistic manner with stress resistance without considering the fact that it is a ratio between two physiological (transpiration and photosynthesis) or agronomic (yield and crop water use) entities (Blum, 2005). Water use efficiency increased in all plasma treatments under haze stress (Figure 5-a). The photosynthetic water-use efficiency is an indicator for the rate of photosynthesis per unit of stomatal conductance and transpiration rate (Santos *et al.*, 2017). As an adaptation to stress condition, plants adjust the relationship between water, transpiration, photosynthesis, and *WUE* through stomatal changes in order to maximize CO<sub>2</sub> assimilation. These mechanisms also prevent water content loss, and thereby reduce levels of tissue damage

(Kafi *et al.*, 2014). According to Chaves and Oliveira (2004), during the initial establishment of stress, the stomatal conductance decreases faster than the photosynthetic assimilation of carbon, resulting in an elevation in *WUE*. Other studies also reported a decrease in *WUE* under water deficit conditions in sugarcane (Cha-Um and Kirdmanee 2008) and maize (Clavijo-Sánchez *et al.*, 2015). Similar results were reported in which the transpiration rate was decreased by 22% as a result of haze stress on wheat plants (Singh and Rao, 1981). They concluded that haze reduces the light reaching the leaf surface and, thereby, decreases the thermal balance of the leaf. Plasma treatment increased the amount of photosynthesis rate and stomatal conductance similar results were obtained increase water use efficiency (Figures 3 and 4). It seems that increasing stomatal conductance increases the transpiration rate, but the plant can have higher levels of photosynthesis in plasma treatments per mole of water. We observed a positive and significant relationship between transpiration rate and *RWC* ( $R^2=78^{**}$ ).

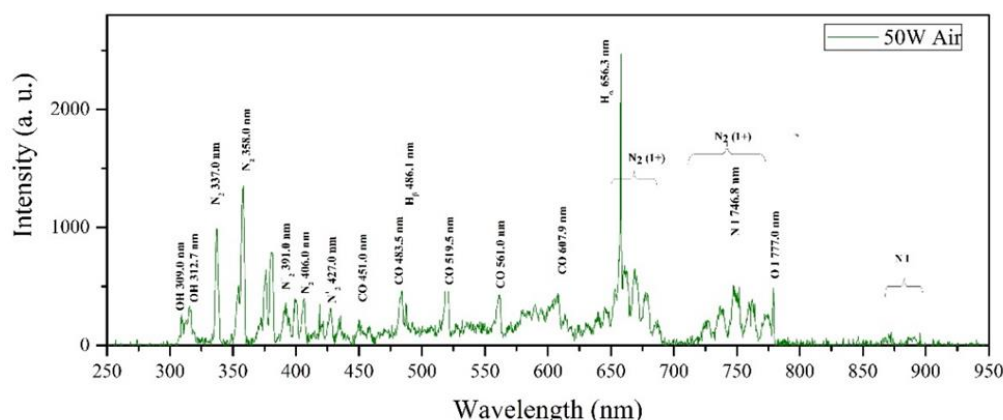
High stomatal conductance and chlorophyll content coupled with higher photosynthetic capacity were responsible for improvement of grain yield in plasma treatments under haze stress condition. The plasma incremental effects on grain yield and spike yield increased up to 58% and 75%, respectively, compared with the control (Figures 8 and 9). The haze stress usually reduces the grain weight during seed filling stage. This is probably due to the reduction of phloem sap movement for the filling of seeds. Reduction in the production of the phloem sap is also associated with the reduction in the photosynthetic process, which is related to stomatal closure (Taiz and Zeiger, 2006). Armbrust (1986) reported a decrease in dry plant weight after one to three days of the haze application, but this was due to the increased frequency of the haze application. Chaurasi *et al.* (2013) reported a decrease in the aboveground biomass of a groundnut crop planted near a cement factory, and grain yield increased with the distance between the factory and the farm.

The plasma incremental effect on the yield was similar to those obtained by other researchers. Some researchers found that the

cold plasma increases yield by altering the shell structure and permeability of the seeds and improving the growth and germination in plants such as *Oriza sativa* (Chen *et al.*, 2012), *Triticum aestivum* (Šerá *et al.*, 2010), and *Solanum melongena* (Zhou *et al.*, 2012). In *Arabidopsis thaliana*, plasma treatment increased the seed weight by 56% in total by 12% in 100-grain weight and by 39% in the number of seeds (Kazunori *et al.*, 2016). In contrast with our result, Fliatovo *et al.* (2011) found that plasma had no significant increase on grain yield of wheat. A more spectacular result was reported by Šerá *et al.* (2008): they achieved three times higher germination rate of *Chenopodium album* seeds after stimulation in a microwave discharge. However, in other studies, no effect of plasma treatment on the germination rate of oat exposed to microwave afterglow (Šerá *et al.*, 2010) and radish seeds treated by RF oxygen discharge (Henselová *et al.*, 2012) was noticed. This wide variation of results among different research groups may be due to diverse plasma treatment conditions, but may also be an indication of the different response of various seed types to plasma exposure.

The seed treatment with plasma can have different effects on the morphological and agronomical characteristics of the seeds, depending on the complex interactions between organic matter and living cells (Šerá *et al.*, 2008). When the seed is exposed to the plasma (Figure 10), oxygen radicals and ions cause surface erosion of the seeds (Stoffels *et al.*, 2008).

The main peaks are monitored in a range of 450-650 nm in the spectra, which consists of CO and hydrogen species. CO emission lines at 451.0, 483.5, 519.5, 561.0, and 607.9 nm and an H emission line at 656.3 nm may represent products of the seed surface erosion process (Figure 10). Several peaks belonging to OH and nitrogen species can also be observed in the spectral range of 250-400 nm. Spectral emissions at 283.4, 309.0, and 312.7 nm indicate the presence of OH species in the RF plasma. Clearly, plasma interaction with material surfaces leads to chemical changes on the surface, thereby increasing seed surface hydrophilicity characteristic.



**Figure 10.** Optical spectra emitted from RF plasma for powers of 50W.

Changes that occur on the seed surface promote the hydrophilic ability of seed and improve water uptake by seed, thus increasing the percentage of the seed reserve mobilization and reducing the percentage of seed reserves. These are some of the important factors in crop productivity determining the initial growth and development of the seedlings (Li *et al.*, 2014). Also, by using cold helium plasma at the booting stage, the treated wheat was taller and had stronger roots as the key factors to increase absorption of water and nutrition (Jiang *et al.*, 2014).

## CONCLUSIONS

The present study indicated that haze stress conditions had several adverse effects on physiological and yield of wheat plants. Cold plasma treatments improved wheat plants tolerance to haze stress by limiting stomatal size, improving stomatal density, increasing intercellular CO<sub>2</sub> concentration, and enhancement of water use efficiency and relative water content. Plasma treatment at 180 seconds ameliorated photosynthesis rate, chlorophyll content, and stomatal conductance by 34, 32, and 93%, respectively, compared with the control. In response to haze stress in plasma treatments at 180 seconds, grain yield and spike yield increased by 58 and 75%, respectively, compared with the control. Finally, it is concluded that cold plasma treatments alleviate haze stress damage in winter wheat. Thus, cold plasma treatment

seems to be a useful approach to protect the wheat plants against the damage caused by haze stress.

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## بهبود فتوستتوز و فرآورده‌های فتوستتوزی گندم پاییزه با استفاده از پلاسماهای سرد در شرایط ریزگرد

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### چکیده

تنش ریزگرد، اثرات مخرب مختلف فیزیکی، شیمیایی و فیزیولوژیکی مختلفی بر گیاهان می‌گذارد. پلاسماهای سرد به عنوان یک فن‌آوری مناسب در افزایش جوانه‌زنی بذر شناخته شده است. این پژوهش به بررسی اثرات پلاسماهای سرد بر کاهش اثرات مخرب ریزگرد بر روی گیاه گندم پاییزه (رقم پیشگام) در گلخانه انجام شده است. ابتدا بذر گندم تحت شارش پلاسماهای سرد هوا ۵۰ وات و در زمانهای ۶۰، ۱۲۰، ۱۸۰ و ۲۴۰ ثانیه قرار گرفت. در گلخانه تحقیقاتی دانشکده کشاورزی دانشگاه تربیت مدرس، شبیه‌سازی ریزگرد با غلظت ۱۵۰۰ میکروگرم بر مترمکعب در مراحل ۱۳، ۴۹، ۶۱ و ۶۹ زادوکس انجام شد. بررسی اثرات پلاسما بر کیفیت و کمیت گندم از طریق اندازه‌گیری شدت فتوستتوز، تراکم روزنه‌های برگ، اندازه روزنه، غلظت دی‌اکسید کربن بین سلولی، میزان تعرق، میزان سبزینگی برگ، محتوای نسبی آب برگ گیاه، هدایت روزنه‌ای و عملکرد دانه تعیین شد. نتایج نشان داد که تفاوت معنی‌داری میان تیمارهای پلاسما و شاهد وجود دارد. به طور مثال، تیمار پلاسما ۱۸۰ ثانیه، میزان فتوستتوز، میزان سبزینگی و هدایت روزنه‌ای را به ترتیب ۳۴٪، ۳۲٪ و ۹۳٪ نسبت به شاهد بهبود بخشید. در پاسخ به تنش ریزگرد پلاسما ۱۸۰ ثانیه، عملکرد دانه و عملکرد سنبله را به میزان ۵۸٪ و ۷۵٪ بهبود بخشید. به طور کلی از این پژوهش نتیجه‌گیری می‌شود که استفاده از تیمار پلاسماهای سرد می‌تواند نقش مهمی در کاهش اثرات تنش ریزگرد در گیاه گندم ایفا کند.